Treatment of nevus using medical tattooing

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1 Introduction

Skin color abnormalities (dyschromatoses) such as pigmented nevi, leukoderma, or senile pigment freckles are often encountered by dermatologists and plastic surgeons. Favorable outcomes can often be obtained by treating these cases using laser technology, which has made significant advances in recent years, or by conventional therapies such as resection, skin transplants, skin peeling, and psoralen ultraviolet A (PUVA) therapy. In some cases, however, these abnormalities resist treatment, causing disappointment to both patient and physician.

Therapy-resistant skin color abnormalities have been treated by means of medical tattooing. Skin color abnormalities can be roughly categorized into: (1) abnormalities in epidermal color, (2) abnormalities in dermal color, and (3) abnormalities in both the epidermal and dermal colors. Senile pigment freckles and nevi are typical epidermal color abnormalities; hemangioma and Ota’s nevus are representative of dermal color abnormalities; and scars typify combined epidermal and dermal color abnormalities. Laser therapy has yielded excellent results when used for treatment of hemangioma. Ota’s nevus is also treated well if the lesion is shallow and allows sufficient laser energy to be applied. However, laser therapy is ineffective for depigmented skin abnormalities (such as leukoderma and scarring) and for nevi that look slightly blue through the skin surface in which the nevus cells are located deep in the dermis. Tattooing injects dye into the skin transplants, skin peeling, and psoralen ultraviolet A.
upper layers of the dermis and thus masks the dermal color and changes the background color of the epidermis. Tattooing is therefore effective for treating depigmented skin.

Medical tattooing uses tattooing techniques to color the skin. It is generally used in the field of plastic surgery to restore the color of the lips and areolae mammae after tissue reconstruction.\(^3\) Currently, selection of appropriate dyes used for medical tattooing are often based on the surgeon’s personal experience, combined with a trial-and-error approach to obtain the desired appearance of the tattooed skin. In our study, a spectrocolorimeter is used to measure the skin color and try to determine the color of the tattooed skin in advance of dye injection, allowing the preparation of an optimal dye mixture for each patient.\(^2\) The spectrocolorimeter is a device to measure the color of a physical object as a spectrum data, and it ensures high reproducibility. Using this device, the reflectance curves of the object are easily obtained without any risk. In this way, it becomes possible to treat color abnormalities using medical tattooing with minimal trial and error. In the present study, a method for predicting the color appearance of any third dye using the result of two trial dyes is proposed by constructing an optical skin model based on the modified Lambert-Beer law. A clinical study of trial tattooing to the skin with loss of pigments has validated the effectiveness of the proposed method in this paper.

2 Tattooing and Optical Skin Models

2.1 Principle of Medical Tattooing

Tattooing has been practiced since ancient times. It permanently colors the skin by injecting a dye subcutaneously. A variety of tools is used for dye injection, in most cases, sharp needles or blades. The injected dye spreads from the skin surface into the subcutaneous tissue immediately after injection. Epidermal cells in the epidermal basal layer are reproduced by cell division, and older cells gradually move to the surface, eventually forming a horny layer before desquamation. For this reason, dye injected shallower than the epidermal basal layer disappears in about a month. Of the dye particles remaining in the subcutaneous cell layer, some are removed by macrophage phagocytosis or transported to deeper tissue by the lymph flow.\(^3\)\(^4\) To predict the color appearance of tattooed skin using arbitrary dye is not an easy task, since it changes with time.\(^5\)

The method used in this study for injecting dye subcutaneously was the blanket method: peeling a thin layer of skin with a razor, injecting the dye, and then restoring the skin layer as shown in Figure 1. The thickness of the skin to be peeled was determined by observing bleeding, then the dye was injected at the interface between the epidermal basal layer and the upper layer of dermis. Tattooing using the blanket method yields results similar to those using a conventional tattooing machine, but the former method requires less time than the latter. A microscopic section shown in Figure 2 demonstrates that the injected dye by the blanket method that remains in the skin is located in the upper layers of dermis.

Since visible light penetrates down to a depth of about 2 mm under the skin surface, only dye particles present less than 2 mm deep under the surface can influence the appearance of skin color.\(^6\) Tattooing forms a layer of dye just below the epidermal basal layer to mask the dermal color [Figure 3(a)] or changes the background color of the epidermis [Figure 3(b)]. The color appearance of tattooed skin is affected by the absorption of light by pigments (melanin and hemoglobin) in the skin and by the scattering of light by cells in the skin. This makes it difficult to predict the color appearance of tattooed skin. The next subsections describe how the light propagation in the skin is mathematically expressed and how it is possible to predict the color appearance of tattooed skin.

2.2 Optical Skin Model Based on the Modified Lambert-Beer Law

In a light scattering and absorbing medium, such as biological tissue, light propagates with complex paths as shown in Figure 4. When the medium consists of multiple layers as shown...
in Figure 5, the absorbance of the reflected light can be expressed by Eq. (1) with the application of the modified Lambert-Beer law to each layer:  

\[
- \log_{10} \frac{I_{\text{out}}(\lambda)}{I_{\text{in}}(\lambda)} = - \log_{10} R(\lambda) = A(\lambda) = \sum_j \varepsilon_j(\lambda) C_j l_j(\lambda) + B(\lambda),
\]

(1)

Here the absorbance \( A(\lambda) \) as the function of the wavelength \( \lambda \) is defined as the logarithm of the inverse of the reflectance \( R(\lambda) \); \( I_{\text{in}}(\lambda) \) and \( I_{\text{out}}(\lambda) \) denote the intensities of light incident on and exiting from the medium, respectively; \( \varepsilon_j(\lambda) \) is the molar absorption coefficient of the absorbing material, \( j \), contained in the layer \( j \) in the medium, and \( C_j \) is its concentration; and \( l_j(\lambda) \) denotes the average optical pathlength in the layer \( j \). The term \( B(\lambda) \) expresses the light component undetected due to scattering. In this study, the term absorbance spectra is used to refer specifically to the absorbance spectra of reflected light. The optical properties of biological tissues vary with wavelength, so the average optical pathlength is a function of \( \lambda \). This is why the color appearance of pigments under the skin is different from its original color. Human skin consists of epidermis and dermis, as shown in Figure 5, and the light absorbing materials in the skin are dominated by melanin in the epidermis and blood (hemoglobin and bilirubin) in the dermis. \(^{5,10} \) Assuming that melanin and blood are the only pigments in the skin, the absorbance spectrum of reflected light can be expressed by Eq. (2),

\[
A(\lambda) = \varepsilon_m(\lambda) C_m l_m(\lambda) + \varepsilon_b(\lambda) C_b l_b(\lambda) + B(\lambda),
\]

where the subscripts \( m \) and \( b \) denote melanin and blood, \( l_m \) is the average optical pathlength within the epidermis containing melanin, and \( l_b \) is the average optical pathlength within the dermis containing blood.

### 2.3 Prediction of the Change in the Color of Skin by Tattooing

Following Eq. (2), the absorbance of the original skin before injection, \( A^{(0)}_{\text{skin}}(\lambda) \), can be expressed by Eq. (3),

\[
A^{(0)}_{\text{skin}}(\lambda) = \varepsilon_m(\lambda) C_m l_m^{(0)}(\lambda) + \varepsilon_b(\lambda) C_b l_b^{(0)}(\lambda) + B^{(0)}(\lambda),
\]

(3)

where the superscript \((0)\) denotes “before dye injection,” and \( l_m^{(0)} \) and \( l_b^{(0)} \) are the average optical pathlengths in the epidermis and dermis before injection, respectively, as shown in Figure 5.

When a layer of pure dye exists separately from the skin, the absorbance of the original dye, \( A^{(0)}_p(\lambda) \), can be expressed by Eq. (4):

\[
A^{(0)}_p(\lambda) = \varepsilon_p C_p l_p^{(0)}(\lambda) + B^{(0)}_p(\lambda),
\]

(4)

where \( \varepsilon_p \) and \( C_p \) are the molar absorption coefficient and molar concentration of the absorber contained in the dye, and \( l_p^{(0)} \) is the average optical pathlength in the pure dye layer.
Here, it is assumed that the pure dye layer has a greater thickness sufficient for negligible transmission as shown in Figure 4.

After dye injection, the skin and dye layers can be modeled as shown in Figure 6, with the average optical pathlengths in the epidermis, dermis, and dye layers expressed by \( I_{p1}^{(1)} \), \( I_{b1}^{(1)} \), and \( I_{p1}^{(0)} \), respectively, with the superscript (1) denoting “after dye injection.” Optical pathlengths in these layers change as the result of dye injection. Assuming that the concentration of melanin and blood remains unchanged by dye injection, the absorbance spectrum of the skin after injection can be given by Eq. (5),

\[
A_p^{(1)}(\lambda) = e_m(\lambda)C_m[I_{m}^{(1)}(\lambda) - I_{m}^{(0)}(\lambda)] + e_b(\lambda)C_b[I_{b}^{(1)}(\lambda) - I_{b}^{(0)}(\lambda)] + [B_{p}^{(1)}(\lambda) - B_{p}^{(0)}(\lambda)] + e_p(\lambda)C_p[I_{p}^{(1)}(\lambda)].
\]  

From Eqs. (3) and (5), the change in the absorbance spectrum is obtained and expressed as the following equation,

\[
\Delta A(\lambda) = A_p^{(1)}(\lambda) - A_p^{(0)}(\lambda) = e_m(\lambda)C_m[I_{m}^{(1)}(\lambda) - I_{m}^{(0)}(\lambda)] + e_b(\lambda)C_b[I_{b}^{(1)}(\lambda) - I_{b}^{(0)}(\lambda)] + [B_{p}^{(1)}(\lambda) - B_{p}^{(0)}(\lambda)] + e_p(\lambda)C_p[I_{p}^{(1)}(\lambda)].
\]

When the dye is injected using the blanket method, most of the dye is injected deeper than the epidermal basal layer, and only a small amount of the dye is carried over to the horny layer to be consequently removed. A fixed amount of dye then remains at a constant depth and occupies a fixed region between the epidermis and dermis. Several types of dye are now considered. These dyes are assumed to have the same light scattering properties as each other, but have different absorption coefficients, \( e_p \), and different concentrations of the absorber, \( C_p \). It can also be assumed that the scattering coefficients of the dyes are much greater than their absorption coefficients as for the case of biological tissues, and that the injected volume of the dyes is the same for all types of dye. So, the average optical pathlengths in the dye layers, \( I_{p1}^{(1)} \), can be the same for all types of dye because \( I_{p1}^{(0)} \) is mostly dependent only on the scattering coefficient when absorption is much weaker than scattering. Then, in Eq. (6), only the values of \( e_p \) and \( C_p \) in the last term on the right-hand side vary depending on the type of dye.

Suppose that there are three types of dye having different colors but the same scattering properties as mentioned above. Each dye is denoted by the subscript \( i (i=1,2,3) \), and has original parameters of \( e_{pi} \), \( C_{pi} \), \( I_{p1}^{(0)} \), \( A_{p1}^{(0)} \), and \( B_{p1}^{(0)} \) in Eq. (4). It can be assumed that the average pathlengths and absorbances of the undetected light are the same for all three different dyes because they are determined mainly by their scattering properties and partly by their absorption properties, i.e.,

\[
I_{p1}^{(0)}(\lambda) = I_{p2}^{(0)}(\lambda) = I_{p3}^{(0)}(\lambda) = I_p^{(0)}(\lambda)
\]

\[
B_{p1}^{(0)}(\lambda) = B_{p2}^{(0)}(\lambda) = B_{p3}^{(0)}(\lambda) = B_p^{(0)}(\lambda).
\]

The absorbance \( A_{p1}^{(0)} \) is then expressed by the following equation:

\[
A_{p1}^{(0)}(\lambda) = e_{pi}(\lambda)C_{pi}I_{p1}^{(0)}(\lambda) + B_{p1}^{(0)}(\lambda) \quad (i=1,2,3).
\]

As a result, the following relationship for the ratio of the absorbance differences between two pure dye layers is obtained:

\[
\frac{A_{p3}^{(0)}(\lambda) - A_{p1}^{(0)}(\lambda)}{A_{p2}^{(0)}(\lambda) - A_{p1}^{(0)}(\lambda)} = \frac{e_{p3}(\lambda)C_{p3} - e_{p1}(\lambda)C_{p1}}{e_{p2}(\lambda)C_{p2} - e_{p1}(\lambda)C_{p1}}.
\]

Now the absorbance changes of the skin before and after the dye injection (tattooing), \( \Delta A_i \) \( (i=1,2,3) \), are considered and expressed by Eq. (10):

\[
\Delta A_i(\lambda) = A_{p1}^{(1)} - A_{p1}^{(0)} = e_{si}(\lambda)C_{si}[I_{si}^{(1)} - I_{si}^{(0)}] + e_b(\lambda)C_b[I_{b1}^{(1)} - I_{b1}^{(0)}] + e_p(\lambda)C_p[I_{p1}^{(1)} - I_{p1}^{(0)}] + [B_{p1}^{(1)}(\lambda) - B_{p1}^{(0)}(\lambda)].
\]

It is again assumed that the average pathlengths and absorbances by the undetected light are the same for all three different dye layers even after injection, i.e.,

\[
I_{p1}^{(1)}(\lambda) = I_{p2}^{(1)}(\lambda) = I_{p3}^{(1)}(\lambda) = I_{p1}^{(0)}(\lambda)
\]

\[
B_{p1}^{(1)}(\lambda) = B_{p2}^{(1)}(\lambda) = B_{p3}^{(1)}(\lambda) = B_{p1}^{(0)}(\lambda).
\]

So the ratio of the differences between \( \Delta A_3 \) and \( \Delta A_1 \) to that between \( \Delta A_2 \) and \( \Delta A_1 \) is expressed as:

\[
\frac{\Delta A_3(\lambda) - \Delta A_1(\lambda)}{\Delta A_2(\lambda) - \Delta A_1(\lambda)} = \frac{e_{p3}(\lambda)C_{p3} - e_{p1}(\lambda)C_{p1}}{e_{p2}(\lambda)C_{p2} - e_{p1}(\lambda)C_{p1}}.
\]
Fig. 7 Picture of dyschromatosis symmetrica hereditaria. Depigmentation is visible in the skin distal to the ankle joint.

Fig. 9 Three dyes, brown (br), white (w), and beige (be), are injected subcutaneously (in squares).
\[
\Delta A_3(\lambda) - \Delta A_1(\lambda) = \frac{A_{p3}^{(0)}(\lambda) - A_{p1}^{(0)}(\lambda)}{A_{p2}^{(0)}(\lambda) - A_{p1}^{(0)}(\lambda)},
\]

This equation leads to Eq. (14):

\[
\Delta A_3(\lambda) = \frac{A_{p3}^{(0)}(\lambda) - A_{p1}^{(0)}(\lambda)}{A_{p2}^{(0)}(\lambda) - A_{p1}^{(0)}(\lambda)} [\Delta A_2(\lambda) - \Delta A_1(\lambda)] + \Delta A_1(\lambda).
\]

The substitution of Eq. (6) by Eq. (14) reduces to Eq. (15):

\[
A_{p3}^{(1)}(\lambda) = \frac{A_{p3}^{(0)}(\lambda) - A_{p1}^{(0)}(\lambda)}{A_{p2}^{(0)}(\lambda) - A_{p1}^{(0)}(\lambda)} [A_{p2}^{(1)}(\lambda) - A_{p1}^{(1)}(\lambda)] + A_{p1}^{(1)}(\lambda).
\]

Equation (14) or (15) expresses the following: When three types of different tattoo dye are prepared, and if the original absorbance spectra of the pure dye layers, \(A_{p1}^{(0)}(\lambda)\), \(A_{p2}^{(0)}(\lambda)\), and \(A_{p3}^{(0)}(\lambda)\), and the absorbance changes (or absorbances) of the skin after injecting dyes 1 and 2, \(\Delta A_1(\lambda)\) and \(\Delta A_2(\lambda)\) \(\text{or}\) \(A_{p1}^{(1)}(\lambda)\) and \(A_{p2}^{(1)}(\lambda)\), are known by colorimeter measurements, the absorbance change (or absorbance) of the skin after injecting dye 3, \(\Delta A_3(\lambda)\) \(\text{or}\) \(A_{p3}^{(1)}(\lambda)\) can be predicted, under the assumption that all the dye types have the same average optical pathlengths, \(l_{pi}(\lambda)\) and \(l_{pi}^{(1)}(\lambda)\), and the same absorbances by undetected light, \(B_{pi}^{(0)}(\lambda)\) and \(B_{pi}^{(1)}(\lambda)\).

2.4 Actual Procedure for Predicting the Color Appearance of Tattooed Skin and Selecting the Best Dye

Based on the formula derived in the above subsection, the actual procedure for predicting the color appearance of the tattooed skin and selecting the best dye is explained in the following. In principle, the spectral data measured by the spectrophotometer are input into the right-hand side of Eq. (15) and the color appearance of the skin injected by a third dye \(\text{[the left-hand side of Eq. (15)]}\) is calculated.

1. Measure the reflectance spectra of the original skin in the areas with and without pigment loss \([R_{\text{skin}}^{(0)}(\lambda)\) and \(R_{\text{norm}}^{(0)}(\lambda)\)] by a spectrophotometer, and transform them to the absorbance spectra \([A_{\text{skin}}^{(0)}(\lambda)\) and \(A_{\text{norm}}^{(0)}(\lambda)\)] by Eq. (1). (Subscript “norm” denotes the skin with a normal color.)

2. Measure the original absorbance spectra of the three dye layers with the greater thickness for negligible light transmission \([A_{p1}^{(0)}(\lambda), A_{p2}^{(0)}(\lambda),\) and \(A_{p3}^{(0)}(\lambda)\)] using a spectrophotometer.

3. Inject the first and second trial dyes into the original skin area with pigment loss. About one month after injection, the color appearance of the tattooed skin reaches a steady state and the absorbance spectra of the tattooed skin \([A_{p1}^{(1)}(\lambda)\) and \(A_{p2}^{(1)}(\lambda)\)] are measured using a spectrophotometer. It is better to use two trial dyes with very different colors.

4. Calculate the absorbance spectrum of the tattooed skin by a third dye \([A_{p3}^{(1)}(\lambda)\)] using Eq. (15).

5. Calculated \(A_{p3}^{(1)}(\lambda)\) is the predicted absorbance spectrum of the tattooed skin by a third dye, and this is compared with the absorbance spectrum of the skin with a normal color \([A_{\text{norm}}^{(0)}(\lambda)\]). If they \([A_{p3}^{(1)}(\lambda)\) and \(A_{\text{norm}}^{(0)}(\lambda)\)] agree well, this third dye is a good candidate for treating the skin with pigment loss.

6. In practice, many dyes are prepared and a database is made by measuring their original absorbance spectra \([A_{p1}^{(0)}(\lambda)\) \((i = 1, 2, 3, \ldots, n)\)] in advance of the trial tattooing. After trial tattooing, the absorbance spectra of the skin tattooed by the prepared dyes \([A_{p1}^{(1)}(\lambda)\) \((i = 1, 2, 3, \ldots, n)\)] can be predicted by the calculation in stage 4, and a dye with the best match of \(A_{p3}^{(1)}(\lambda)\) with \(A_{\text{norm}}^{(0)}(\lambda)\) is selected.

The total period of time for treatment is governed by the time it takes for the tattooed skin to reach a steady state after injection. Injection of the trial dyes by the blanket method requires only about 15 min, and the main injection of the selected dye about 30 min to one hour. It takes about one month for the tattooed skin to become steady after injection. So, at least two months are required, one month for the trial tattooing and another month for the main tattooing. The time for measurements using a colorimeter takes only a few minutes, and calculation using Eq. (15) will require less than 10 min when it becomes routine practice. The greatest risk, if any, is an allergic reaction to the dyes. However, the existing dyes have long been used clinically. There have been no reports of problems with the dyes used in this study. Only very rarely does tattooing itself induce keloid.

3 Validation of the Method by a Clinical Trial Tattooing

The method described above has been validated by a clinical trial tattooing. The patient was a 49-year-old male with dyschromatosis symmetraca hereditaria. His chief complaint was depigmentation on his dorsum manus and dorsum pedis on both sides as shown in Figure 7 (see Color Plate). He consulted our clinic about the possibility of reducing the difference in color between the normal and affected areas by medical tattooing. He had previously undergone PUVA and transplantation of cultured skin, but the depigmentation of his feet had not been corrected. The clinical trial by the method proposed in this study was performed on the patient. He gave his informed consent after detailed explanation of this method. Then, following the usual procedure for tattooing therapy using the blanket method, the small areas \((10 \times 10 \text{ mm})\) of depigmented skin on the backs of his feet were tattooed using three trial dyes. One month after the trial dye injection, measurements using a spectrophotometer were conducted on the tattooed areas and their absorbance spectra were obtained.

A spectrophotometer (Minolta CM-2022) shown schematically in Figure 8 was used in this study. This colorimeter consists of an integrating sphere, a xenon lamp as the light source, and two light detectors for the light source and for light reflected from the skin or other materials. The integrating sphere plays the important dual role of generating a diffusely illuminating light source and, at the same time, collecting all the diffusely reflected light from the skin or other.
materials. The integrating sphere has an inner diameter of 52 mm and all the components are enclosed in a handy cylindrical container. It conforms to the standard specified in Section C of JIS (Japanese Industrial Standards) Z8722 with a measuring wavelength range from 400 to 700 nm and with a wavelength interval of 10 nm. Three separate measurements with this device were repeated for each measuring site, and the average absorbance spectrum was obtained. All measurements for one condition were performed by the same researcher to minimize errors due to differences in compression of the skin and inclination of the colorimeter, which were the two major error sources of this contact type colorimeter.

No special qualification is required by medical doctors for tattooing treatment. If no anesthesia is needed, tattooing itself requires no qualification. No special technique is required for measurement using a colorimeter. A colorimeter costs about 10,000 U.S. dollars and a tattooing machine about 2,000 U.S. dollars.

Three types of tattoo dye were prepared, white, beige, and brown, by mixing the dyes approved by the Food and Drug Administration, USA originally attached to a tattooing machine (Permark, USA). These three dyes contained titanium oxide as the major scattering material with a concentration of between 37% and 42% in weight as shown in Table 1, allowing the light scattering properties of all three dyes to be regarded as the same.

As mentioned in Sec. 2.4, trial injection should be conducted using two dyes first, and a third dye should be injected when the colors of the tattooed skin reach a steady state about one month after injection. But in this study, all three dyes were injected at the time of the trial injection. The depigmented area on the back of the patient’s foot was treated using these three dyes as shown in Figure 9 (see Color Plate). In this study, white and brown dyes were considered as the two trial dyes, and the beige dye as the third one. The absorbance spectra of the tattooed skin by the three dyes were measured, and the predicted absorbance spectrum of the third dye using Eq. (15) was compared with the measured one to validate the method proposed in this study.

## 4 Results and Discussion

Figure 10 shows the absorbance spectra of the three pure dye layers. The subscripts $w$, $be$, and $br$ denote the white, beige,

### Table 1

<table>
<thead>
<tr>
<th></th>
<th>Titanium oxide</th>
<th>Indian red</th>
<th>Yellow iron oxide</th>
<th>Glycerin</th>
<th>IPA</th>
<th>Water</th>
</tr>
</thead>
<tbody>
<tr>
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<td>42</td>
<td>0</td>
<td>0</td>
<td>18</td>
<td>31</td>
<td>9</td>
</tr>
<tr>
<td>beige</td>
<td>39</td>
<td>1</td>
<td>2</td>
<td>28</td>
<td>25</td>
<td>5</td>
</tr>
<tr>
<td>brown</td>
<td>37</td>
<td>2</td>
<td>3</td>
<td>28</td>
<td>25</td>
<td>5</td>
</tr>
</tbody>
</table>

(weight %)

![Fig. 10 Absorbance spectra of the three types of pure dye layers, A(0)br, A(0)be, and A(0)w.](https://www.spiedigitallibrary.org/journals/Journal-of-Biomedical-Optics)

As mentioned in Sec. 2.4, trial injection should be conducted using two dyes first, and a third dye should be injected when the colors of the tattooed skin reach a steady state about one month after injection. But in this study, all three dyes were injected at the time of the trial injection. The depigmented area on the back of the patient’s foot was treated using these three dyes as shown in Figure 9 (see Color Plate). In this study, white and brown dyes were considered as the two trial dyes, and the beige dye as the third one. The absorbance spectra of the tattooed skin by the three dyes were measured, and the predicted absorbance spectrum of the third dye using Eq. (15) was compared with the measured one to validate the method proposed in this study.

Figure 11 shows the absorbance spectra of the skin before and after tattooing. Pre-tattoo skin is the absorbance spectrum of the depigmented skin before dye injection, and A(1)br, A(1)be, and A(1)w are those after injection of the three dyes, respectively. The absorbance spectrum of the normal skin is also shown for reference.

![Fig. 11 Absorbance spectra of the skin before and after tattooing. Pre-tattoo skin is the absorbance spectrum of the depigmented skin before dye injection, and A(1)br, A(1)be, and A(1)w are those after injection of the three dyes, respectively. The absorbance spectrum of the normal skin is also shown for reference.](https://www.spiedigitallibrary.org/journals/Journal-of-Biomedical-Optics)
and brown tattoo dyes, respectively. In the measurements of the absorbance spectra of pure dye layers, thick layers of dye were prepared on a sheet of white paper to ensure negligible transmitted light, and their original absorbance spectra were measured using the spectrophotometer. Because these dyes are light-scattering materials, the measured absorbance spectra are not pure absorption spectra but contain characteristics of both absorption and scattering. Figure 11 shows the absorbance spectra of normal skin, of depigmented skin before tattooing, and of depigmented skin after injection of the three dyes using the blanket method.

Figure 12 shows the differences in the absorbance spectra after and before injection at the depigmented skin areas, $\Delta A_{be}$, $\Delta A_{br}$, and $\Delta A_w$. The value of $\Delta A_i$ for the white dye is seen negative over the entire wavelength range, and that of the beige dye is negative in the longer wavelength range. The reason for these negative values is that the white and beige dyes have a higher scattering coefficient than that of the dermis. As the result of the higher scattering in the dye layer, the optical pathlength in the dermis after dye injection becomes shorter than that before injection, and absorption of light by blood decreases in proportion. White dye has very low absorption as shown in Figure 10; therefore, the reflected light intensity becomes larger after dye injection resulting in the negative values of $\Delta A_w$ over the whole wavelength range. Beige dye has lower absorption only in the longer wavelength range and thus $\Delta A_{be}$ is negative in that range as shown in Figure 12.

If the dyes 1, 2, and 3 in Eq. (15) are assumed to be the white, brown, and beige dyes, respectively, the absorbance spectrum of the skin injected with the beige dye can be predicted from the measured absorbance spectra of the skins trially injected by the white and brown dyes as well as the original absorbance spectra of the three pure dye layers. Figure 13 shows measured and predicted absorbance spectra of the beige injected skin. They agree very well, and the color difference expressed by the $L^*a^*b^*$ color expression system was as small as 3.67, which was calculated using the measured and predicted spectra. When the two spectra were converted to colors on a CRT display, the predicted color was very close to the actually measured color and the color difference was not observable to the naked eye.

These results indicate that the color appearance of dyes after injection can be successfully predicted using the absorbance measurements of trial injection of two dyes. In particular, by preliminary measurements of the differences in the absorbance spectra between pre- and post-injection of two dyes, which have very different absorbance spectra from each other, the absorbance spectra of the skin injected by a dye whose absorbance spectrum lies between those of the previous two dyes can be fairly accurately predicted. Equation (15) states that the absorbance spectrum of the skin injected by a third dye can be obtained by interpolation between those by the two trial dyes with the proportionality factor using those of the pure dye layers.

The absorbance spectrum of the skin injected by the third (beige) dye has disagreement with that of the normal skin at some wavelengths in the whole wavelength range. This is because the third dye was not prepared using Eq. (15) but prepared as an arbitrary mixture of white and brown dyes based on the experience of a medical doctor at the time of the
trial tattooing. If many types of dye mixtures are prepared, a third dye, which provides the best match for the color of the tattooed skin with that of the normal skin, will successfully be found.

The most important assumption in the derivation of Eq. (14) or (15) is that the optical pathlengths $l_{pi}^{(0)}$, $l_{pi}^{(1)}$, $B_{pi}^{(0)}$, and $B_{pi}^{(1)}$ are the same for all the dyes 1, 2, and 3. Figures 10 and 12, which compare the absorbances of the skin injected by the three dyes, demonstrate that this assumption is not valid, because a difference in the absorbance means differences in the absorption coefficients among the dyes, and the differences in the absorption coefficients lead to differences in the optical pathlength even if the scattering coefficients are the same. The validity of this assumption can be checked by comparing the left- and right-hand sides of Eq. (13), and they are compared in Figure 14. The agreement of the two curves in Figure 14 can be either good or bad. Nevertheless, a good agreement of the predicted spectrum with the measured one in Figure 13 mostly validates the assumption for the application of medical trial tattooing proposed in this study.

In this study, only three dyes were prepared and there was still some difference in color between the normal skin and the skin injected with the third dye. If more dyes are prepared as candidates for the third dye and a database of their absorbance spectra is ready, the third dye providing the best match for the normal skin color will be easily found and no more trial injections will be required.

If dyes can thus be prepared to accurately reproduce the target skin color, it will be possible to carry out medical tattooing with a minimum volume of dye, and without the need for repeated tattooing to approach the target color, thereby reducing the stress on the patient undergoing this therapy. Moreover, the results in this study suggest that the proposed method may be applicable not only in cases of leukoderma but also for other skin color abnormalities such as intradermal nevi and reconstructed nipples.

5 Conclusions
To improve the performance of tattooing therapy for skin color abnormalities, an optical model based on the modified Lambert-Beer law was created for analyzing light propagation in the skin. Using this model, a formula to predict the color appearance of tattooed skin by dye injection was derived.

By applying this formula to the trial injection of two different dyes, it was possible to predict the color appearance of the skin injected by a third dye. The predicted absorbance spectrum of the tattooed skin was very close to the actually measured one. Based on the results of this study, this method can be utilized to predict what skin color will be obtained by dye injection, thus contributing to improvements in the usability and effectiveness of medical tattooing.

References