Optical characteristics of human skin with hyperpigmentation caused by fluorinated pyrimidine anticancer agent

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Abstract: The fluorinated pyrimidine anticancer agent has several side effects that degrade the quality of life of patients, including hyperpigmentation. Hyperpigmentation differs in color from common pigmentation such as a suntan, giving rise to dramatic skin appearance changes. In this study, we measured the optical properties of the skin of patients with hyperpigmentation by using the reflection spatial profile method (RSPM). The absorption coefficient in hyperpigmentation increased ~1.5–2.5 times and pheomelanin significantly increased compared to the normal skin. In addition, the scattering coefficient of skin with hyperpigmentation was about 65.9–76.5% of that of normal skin.

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1. Introduction

Anticancer drug therapy is used against tumors that have spread widely in the body of the patient. In recent years, molecular targeted drugs and cytotoxic drugs have been developed. However, these drugs have many side effects, including skin disorders such as acne and hyperpigmentation different from typical pigmentation such as that of suntanned skin. In this study, we focused on hyperpigmentation, which is one of the significant side effects of the fluorinated pyrimidine anticancer agent (5-FU, capecitabine, S-1). This fluorinated pyrimidine anticancer agent is used for several types of cancers, such as colon, stomach, esophagus, pancreas, breast, and cervical cancer in both palliative and adjuvant settings.

The hyperpigmentation caused by the treatment drug dramatically discolors the skin (Fig. 1). There is no danger to life, but patients do not want to be seen due to the degraded appearance of their skin. The hyperpigmentation reduces their Quality of Life (QOL), leading some patients to opt to cease anticancer drug treatment.

Fig. 1. Photograph showing (a) face and (b) hand depicting a hyperpigmentation appearance caused by fluorinated pyrimidine anticancer agent, notably (a) entire face and (b) interphalangeal joints of hand with discoloration of the skin.
Hyperpigmentation recovers in many cases upon discontinuing chemotherapy administration. However, during the continuation of chemotherapy with these drugs there is no relief. Therefore, psychological care and concern about external appearance need to be considered to maintain the QOL of patients. As a countermeasure other than chemotherapy cessation, patients and psychology therapists try to hide hyperpigmentation with cosmetics. However, since hyperpigmentation has a unique color, different from that of typical pigmentation such as that of suntanned skin, there are no cosmetic products suitable for concealing it.

The skin is a semitransparent medium that scatters and absorbs radiation (light). Its appearance is determined by absorption due to melanin and hemoglobin and scattering due to the organic structures, such as mitochondria and collagen fibers [1]. For example, suntan makes the skin darker due to melanin absorption, which increases under the influence of ultraviolet radiation. However, it has not been clarified what causes the unique appearance of hyperpigmentation.

Case reports of hyperpigmentation have been presented in several papers. Lal [2] reported on cases of hyperpigmentation of both the palms and soles, and the dorsa of the hands and feet of a 55-year-old woman using Capecitabine in breast cancer treatment. Sanz-Sánchez et al. [3] reported on hyperpigmentation in a 75-year-old man who had undergone right hemicolectomy followed by neoadjuvant cycles of chemotherapy with 5-FU. Sakuramoto et al. [4] stated that among 517 patients who used S-1, hyperpigmentation was observed in 241 people (46.6%). In these investigations, hyperpigmentation was considered to be caused by fluoride pyrimidine anticancer agents, but hyperpigmentation has not been analyzed in detail, nor have its optical characteristics been clarified.

Optical property measurement is an effective means of studying skin with various symptoms such as changes in appearance due to skin disorders. Hsu et al. [5] non-invasively measured the optical properties of keloid scars remaining as a result of abnormal wound healing as well as the normal optical properties of skin using a method called diffuse reflectance spectroscopy (DRS). They concluded that the results suggested that DRS has potential as an objective technique with which to evaluate keloid scar severity and that it may be useful for tracking the longitudinal responses of scars to various types of therapeutic intervention. Sharif et al. [6] measured skin with port wine stain (PWS), which is a type of progressive vascular malformation occurring in 0.3–0.5% of the population, using spatial frequency domain imaging (SFDI). They clarified the increase of the total hemoglobin value and decrease of the oxygen saturation degree of the tissue and showed that the changes in tissue structure can be determined based on the scattering coefficient. Zhang et al. [7] presented an initial study on applying genetic algorithms to retrieve the optical properties of human skin using visual reflectance spectroscopy to assist clinical treatment and diagnosis. They concluded that their algorithms can easily distinguish melanin concentrations for different skin types, such as in individuals with PWS, and thus leading to a reasonable understanding of the blood volume fraction.

The authors of this paper have been developing methods of measuring the optical properties of skin and corresponding measurement devices. In addition, by measuring the optical properties of the normal skin of many healthy Japanese people over a wide range of age groups, we obtained data that can be compared to those obtained from patients with skin exhibiting symptoms such as hyperpigmentation. In this study, in order to understand the optical characteristics of hyperpigmentation, we measured the optical properties of the skin of Japanese individuals with hyperpigmentation and analyzed what causes the unique appearance of skin with hyperpigmentation which were compared with the normal skin.

2. Optical properties of human skin

In human skin, when radiation (light) is incident on the skin from the outside, less than 5% of it is reflected from the surface, while the rest penetrates into the skin. Radiation that
penetrates into the skin is absorbed and changed into heat or scattered by intracellular tissues and cell interfaces before exiting the skin again. The amount of outgoing radiation is 20%–50% in the wavelength range of visible light. Human skin can be assumed to be a continuous medium that scatters and absorbs radiation, and radiation transfer inside the skin is often described using the following radiative transfer equation [8,9]:

\[
\frac{1}{\beta} \frac{dI(s, \Omega)}{ds} = -I(s, \Omega) + \frac{\omega}{4\pi} \int_{s' \in \Omega} p(\Omega' \rightarrow \Omega) I(s, \Omega') d\Omega',
\]

where \( I \) is the intensity of the radiation, \( \Omega \) is a unit vector representing the propagation direction of the radiation, and \( s \) is the coordinate along this direction. Equation (1) includes three optical properties: the extinction coefficient \( \beta \) and albedo \( \omega \) which are expressed in terms of the absorption coefficient \( \mu_a \) and scattering coefficient \( \mu_s \), and scattering phase function \( p \). The absorption coefficient \( \mu_a \) and the scattering coefficient \( \mu_s \), were calculated using Eqs. (2) and (3):

\[
\beta = \mu_a + \mu_s,
\]

\[
\omega = \frac{\mu_s}{\beta}.
\]

In the wavelength range of visible light, absorption of light by human skin is mainly induced by eumelanin, pheomelanin, oxyhemoglobin (HbO₂), and deoxyhemoglobin (Hb) [10,11]. Also, the scattering coefficient is affected by the structural organization. We measured these optical properties to analyze hyperpigmentation of the skin.

3. Measurement of the optical properties of skin

3.1 Principle of the estimation method

Figure 2 shows an outline of the reflection spatial profile method (RSPM [12,13]) used to estimate the optical properties in this study. In this estimation method, a structured light is used to irradiate the skin, resulting in an alternating pattern of irradiated and non-irradiated sections of skin, and the spatial profile of the reflected light is measured. In a scattering and absorbing medium such as skin, the penetrating light propagates through repeated or multiple scattering and is measured as reflected light, not only from the portions irradiated by the incident light, but also from the non-irradiated portions. In such a situation, if the extinction coefficient of the skin is considered to be small, then the light tends to spread, and a strong reflected light is measured from the non-irradiated part. Meanwhile, if the albedo is large, the radiation energy absorbed internally decreases, resulting in the measurement of intense light from both the irradiated and non-irradiated parts. These features in turn imply that the spatial profile of the reflected light depends on the optical properties inside the skin. Thus, based on the spatial profile of the reflected light intensity, inverse analysis can be used to estimate the optical properties of the skin.

![Fig. 2. Reflection by surfaces exposed to structured light; (a) opaque material (e.g., a metal), (b) semitransparent material (e.g., human skin), (c) reflection spatial profiles of the opaque material (blue line) and semitransparent material (red line).](image)
3.2 Numerical model for the inverse method

In this section, model of the skin for the numerical analysis is first explained. Then, to reproduce the principle described in the Section 3.1, we describe conditions for the structured irradiation and calculation method of the reflected light for the inverse analysis using the skin model along with the limitations.

The radiation propagation in human skin can be calculated numerically using Eq. (1) by Monte Carlo method [8], where the skin tissue is assumed as homogeneous media. Here, we give a brief description of the model used in analysis (for details refer to [12,13]). The analysis domain is a cuboid, and the sides are the periodic boundaries; thus, the numerical model replicates periodic structured irradiation, as shown in Fig. 2. Here, we further assume the internal structures of the skin negligibly affect the reflection characteristics as the skin has enough optical thickness defined as the geometric thickness multiplied by the extinction coefficient and is set to be 20. Moreover, the boundary conditions for the bottom surface are chosen so that they have zero reflection coefficient and the radiation that is reaching it to be totally absorbed. The skin surface is assumed to be smooth, and the reflection at the surface is calculated by the Fresnel relations. The refractive index \( n \) of the skin is in the range of 1.35 to 1.55 [14,15], which varies depending on moisture content. Therefore, the refractive index of the skin is assumed to be 1.5.

The calculated intensity profiles of the radiation reflected by the skin are presented in Fig. 3. These results are normalized by the intensity of the incident radiation in the irradiated sections divided by \( \pi \). Here, the skin is exposed to structured light with a 2 mm pitch, where the pitch \( L \) is defined as indicated in the middle sketch of Fig. 2 with equal extents for the irradiated and non-irradiated sections of the structured light. The angle of irradiation is 30°, and the reflected radiation is measured from the direction normal to the surface (0°). As shown in Fig. 3, the calculated reflected radiation intensities exhibit sinusoidal profiles, which resemble the intensity profiles of the incident radiation.

![Fig. 3. Calculated reflected radiation intensity profiles.](image)

To investigate the relationship between the optical properties and the profile of reflected radiation, (a) average intensity (Fig. 4(a)) and (b) average deviation (Fig. 4(b)) were calculated using Eqs. (4) and (5), respectively:

\[
\bar{T} = \frac{\int_{y}^{y+L} i(y)dy}{L}, \quad (4)
\]

\[
\bar{\sigma} = \frac{\int_{y}^{y+L} \left| i(y) - \bar{T} \right|dy}{L}, \quad (5)
\]
where $L$ is the pitch of irradiated structured light. Figure 4(a) shows that the average intensity as a function of albedo $\omega$ over a wide range of extinction coefficients $\beta$ from 1 to 50 mm$^{-1}$ and can be seen it depends only on the albedo and not affected by changing $\beta$. On the other hand, Fig. 4(b) shows the average deviation of the reflected intensity as a function of extinction coefficient $\beta$ for four different albedo values $\omega$ of 0.9, 0.94, 0.98 to 1.0. This indicates the deviation depends only on the extinction coefficient once the albedo is derived or determined.

Based on our modelling, when skin is irradiated using the structured illumination having the same structure as that of the model, then the reflected radiation profile can be measured. From the measured profiles, the average intensity and the average deviation can be calculated. At first, the albedo was estimated from the measured average intensity and the estimated albedo was then used to estimate the extinction coefficient. The coefficient was calculated based on the measured average deviation for the intensity. Hence, the optical properties can be estimated from the measured results by inverse analysis.

Fig. 4. Relationship between optical properties and the profile of reflected radiation: (a) average intensity and (b) average deviation of the calculated reflected radiation profiles.

This numerical model is limited to homogeneous media because the reflected radiation profile is simulated on the assumption that human skin has homogeneous optical properties. In fact, the skin possesses a complex structure in the depth direction, with epidermis being composed of layers such as stratum corneum, stratum spinosum, and dermis, which in turn contains layers such as the papillary stratum and reticular layer. However, when a person sees the human skin, the eye recognizes the energy of reflected radiation from the skin, not by distinguishing its complex structure. To understand the how hyperpigmentation dramatically changes the appearance of the skin, it is necessary to investigate the change in reflected radiation energy caused by the optical properties. Even assuming homogeneous optical properties, we can investigate the change of reflected radiation and its phenomenon corresponding to the change of appearance of the skin. As for the visible wavelength, radiation can reach the depth of the dermis. In order to have radiation reach the depth of dermis, the pitch $L$ of irradiated radiation was determined to be 2 mm. When the pitch is changed, the average optical properties also change [16]. If this pitch is short (i.e., on the order of micrometers), only a shallow region (e.g., the stratum corneum of the epidermis) is measured.

3.3 Scattering phase function

When the scattering phase function is assigned, the relationship between the optical properties and the profile of reflected radiation shown in Fig. 4. For the inverse analysis to estimate the extinction coefficient and albedo, the scattering phase function must be assigned. The phase
function used in this analysis was taken from a study conducted by one of the authors, Naito et al. [17], which is shown in following Eqs. (6) and (7):

$$p_{HG}(g) = \frac{(1 - g^2)}{(1 + g^2 - 2g \cos \theta)},$$  \hspace{1cm} (6)

$$p(0^\circ \leq \theta < 51^\circ) = 0.89 \cdot p_{HG}(g = 0.77)$$

$$p(51^\circ \leq \theta \leq 180^\circ) = 0.47 \cdot p_{HG}(g = 0.36),$$  \hspace{1cm} (7)

where $\theta$ is the polar angle. For the scattering phase function, $p$, they adopted the Henyey-Greenstein (HG) function with the anisotropic factor $g$, as shown Eq. (6). Furthermore, they concluded that a single HG function using only one asymmetry factor cannot sufficiently approximate their measured results. Therefore, they described it using two HG functions as shown in Eq. (7). Van Gemert et al. [14] reported that the anisotropic factor $g$ of human skin is between 0.7 and 0.9; Hsu et al. [5] used 0.8 for the anisotropic factor of scattering phase function in their measurements. As the scattering phase function shown in Eq. (7) is close that of these other studies, we used Eq. (7) as a putative value in our measurements.

3.4 Measurement instrument

We developed an instrument for measuring the optical properties based on the RSPM in a previous study [13]. A schematic of the instrument is depicted in Fig. 5. A halogen lamp (HLX-64620, Osram GmbH) is used as the radiation source. To produce the structured light, the radiation from the lamp was passed through a multi-slit mask. Then, the radiation was projected onto the skin by an extended projection lens (HF16SA-1, Fujifilm Corp.). The radiation intensity profile is captured by a cooled charge-coupled device (CCD) camera (BU-51LN, Bitran Corp.). Because the irradiation angle is $30^\circ$ and the reflected radiation is measured from the direction normal to the surface ($0^\circ$), the measured reflected radiation does not include specular reflection when the skin is approximately planar. Although, there is some specular reflection measured due to the roughness of the skin surface, its energy is very small compared to the energy reflected from the inside [18]. Therefore, the influence inside the skin can be measured using this instrument.

![Fig. 5](image)

**Fig. 5.** A schematic of the instrument for the in vivo measurement of skin: 1. Halogen lamp, 2. multi-slit mask, 3. extended projection lens, 4. in vivo human skin, 5. aperture, 6. collimator lens, 7. diaphragm, 8. diffraction grating, 9. Charge-coupled device (CCD) camera. The insets show the (left) multi-slit mask used for producing structured illumination with pitch $L$ being 2 mm and (right) a sample spectral profile from the CCD camera.
Since this optical setup includes a spectroscopic system, the spectral information of the radiation intensity profile is also recorded using the CCD camera. A sample image of the spectral image captured by the CCD camera is shown in Fig. 5. The vertical direction shows the position, while the horizontal direction represents the spectral dependence of the profile. Therefore, the RSPM can be used to estimate optical properties by taking a single picture. Since this measurement method involves estimation of the average optical properties within the measurement area shown in Fig. 5, the influence of the local inhomogeneities of the skin is considered to be small.

In order to compare the experimental results with the inverse analysis, calibration of the experimental system was required. Firstly, the intensity of reflected radiation using a white diffuse reflectance plate whose isotropic hemispherical reflectance is certified (Labsphere Inc., Spectralon Diffuse Reflectance Standards SRM-99) was measured. Then, the ratio of the reflected profile of radiation intensity $i$ of the human skin was measured using Eq. (8):

$$
\frac{i}{i_0} / \rho
$$

where, $i_0$ is measured intensity of reflected radiation for human skin, $i_0$ is measured intensity of reflected radiation for the Diffuse Reflectance Standard plate, and $\rho$ is reflectivity of the Diffuse Reflectance Standard plate. From the measured $i$, we can estimate the albedo and the extinction coefficient by calculating the average intensity and average deviation.

4. Subjects

4.1 Japanese people with normal skin

In our previous study, we measured the optical properties of the skin of 198 Japanese people with normal skin [19]. The normal Japanese skin typically classified as III to IV according to the Fitzpatrick Skin Type [20]. The ages and genders of the subjects are summarized in Table 1. Here, $N_n$ refers to the number of subjects. These measured data were compared with those obtained from patients with hyperpigmentation caused by anticancer agents in this study. Ref. 14 reports the effects of age and gender in individuals with normal skin, and they found that the effects of age and gender were small compared to the hyperpigmentation. In order to show the range of the optical properties of the normal skin with a large population, all age groups were used as subjects of normal skin for comparison.

| Age   | Male | Female |
|-------|------|--------|
| 20–59 | 71   | 90     |
| 60–69 | 14   | 5      |
| 70–89 | 7    | 11     |

4.2 Japanese people with hyperpigmentation

In this study, a total of 34 patients (15 males, 19 females) were recruited at the National Cancer Research Center Central Hospital in Japan based on certain criteria: that one month or more had passed since the administration of a fluorinated pyrimidine anticancer agent (S-1, Capecitabine, 5FU) and that, according to the Common Terminology Criteria for Adverse Events version 4.0, they were judged to have excess skin pigmentation of Grade 2. Grade 2 corresponds to pigmentation occurring within an area occupying more than 10% of the body surface area for patients aged 20 years or older who experience social and psychological effects. The protocol was approved by the facility review committee of the National Cancer Center (No. NCC2015-360), and written informed consent was obtained from all of the subjects before measurement. Table 2 shows the numbers of subjects with hyperpigmentation caused by the anticancer agent according to their ages and genders.
Table 2. Numbers of Subjects with Hyperpigmentation (Nh = 34)

| Age  | Male | Female |
|------|------|--------|
| 20–59| 5    | 5      |
| 60–69| 5    | 8      |
| 70–89| 5    | 6      |

As shown in Fig. 1, the hyperpigmentation has been possibly found to show strong symptoms in the face and hand [21,22]. For this reason, in order to understand the optical characteristics of hyperpigmentation, we chose cheek with a large hyperpigmentation area where measurement can be performed easily. Moreover, we expect because of less facial hair, we suspect the optical properties measurement would be much less affected. Further we could not find large gender based differences in our measurements.

5. Results and discussion

5.1 Scattering coefficient measurement results

Figure 6 presents the scattering coefficients measured for normal cheek skin and cheek skin with hyperpigmentation.

![Fig. 6. Scattering coefficients of normal cheek skin and cheek skin with hyperpigmentation.](image)

When the average value of the scattering coefficient of skin with hyperpigmentation was divided by that of the normal skin at each wavelength, the scattering coefficient for the skin with pigmentation decreased to 65.9–76.5%. Before discussing the decrease of scattering coefficient, we consider the difference in scattering coefficients between skin layers such as the epidermis and dermis.

In this study, we measured the scattering coefficients of skin by assuming the skin to be homogeneous. Strictly speaking, however, human skin has a layered structure, and the scattering coefficient is reported to be lower in the dermis than in the epidermis. Salomatina et al. [23] measured the differences between layers of skin in vitro and showed that the extinction coefficient of the epidermis is about 1.6 times larger than that of the dermis when the wavelength is 600 nm. We also measured the differences between the layers of skin in vivo and found that the surface layer of the skin has a scattering coefficient twice as large as that of the dermis [16]. Based on this knowledge, we discuss below the decrease of the scattering coefficient caused by the anticancer drug treatment.

In normal skin, turnover constantly occurs. That is, new cells are formed at the stratum basale between the epidermis and dermis and go up to the skin surface while forming the epidermis and stratum corneum. Finally, they drop from the skin surface. On the other hand, in the skin of patients treated with the anticancer drug, since treatment with anticancer drugs
can induce cytotoxic activity and cell death by angiogenes inhibition [24], it is considered that new cells are not formed easily. Consequently, the epidermis can become thinner because the dropping of the skin surface occurs daily due to the treatment [25]. Possibility of such thinning has been suggested in animal studies of Writh et al. [26]. They investigated the effect of the 5-FU on the thymidine labelling index, the mitosis rate and the epidermal thickness of skin tissue using 80 male guinea pigs. They concluded that the thickness of epidermis decreases continually after a treatment with the 5-FU. Thus due to the possibility of decreasing thickness of the epidermis, the scattering coefficient of the dermis, which is smaller than that of the epidermis, could mainly contribute to the measured scattering coefficients of the skin of the patients with hyperpigmentation as shown in Fig. 6.

It should be noted that reduction of the scattering coefficient of the skin affects its appearance. Even if the absorption coefficient remains constant, as the scattering coefficient decreases, the reflection of light by the skin decreases, resulting in a darker appearance. This effect occurs because the travel path of the light increases, so more light is absorbed within the skin. This phenomenon may cause the unique color of the skin of patients undergoing anticancer drug treatment, as well as the absorption coefficient changes described in Section 5.2.

5.2 Absorption coefficient measurement results

Figure 7 shows the measured absorption coefficients of normal cheek skin and cheek skin with hyperpigmentation.

![Fig. 7. Absorption coefficients of normal cheek skin and cheek skin with hyperpigmentation.](image)

The absorption coefficient is increased by about 1.5–2.5 times in the patients with hyperpigmentation caused by the anticancer agent. The factor of this change is discussed in greater detail in Section 5.3.

5.3 Absorption coefficient analysis

We analyzed the contributions of different absorbing substances namely, eumelanin, pheomelanin, oxy- and deoxy hemoglobin to the absorption coefficients of the skin. When the molar absorption coefficients $\varepsilon_{\text{eumelanin}}$, $\varepsilon_{\text{pheomelanin}}$, $\varepsilon_{\text{HbO}_2}$ and $\varepsilon_{\text{Hb}}$ have the values shown in Fig. 8, the measured absorption coefficient of skin $\mu_{a,\text{skin}}$ can be expressed using the following equation:

$$\mu_{a,\text{skin}} = M_{\text{eumelanin}} \times \varepsilon_{\text{eumelanin}} + M_{\text{pheomelanin}} \times \varepsilon_{\text{pheomelanin}} + M_{\text{HbO}_2} \times \varepsilon_{\text{HbO}_2} + M_{\text{Hb}} \times \varepsilon_{\text{Hb}}, \quad (9)$$
where $M_{\text{substance}}$ represents the molar concentration of each substance. Using a non-negative least squares method [27] with MATLAB software (MATLAB R2018b, The MathWorks Ltd.) for the fitting algorithm, $M_{\text{substance}}$ can be estimated so that $\mu_{a,\text{skin}}$ coincides with the measured absorption coefficients. As shown in Figs. 6 and 7, the optical properties of human skin have large standard deviation and are different for each individual. Thus, in our research, fitting algorithm was applied to each measurement result of the absorption coefficient individually. After the application of the individual fitting, the average value of the molar concentrations for the substances, which are eumelanin, pheomelanin, HbO$_2$ and Hb was calculated. The estimated molar concentrations are summarized in Table 3, and the fitting curves obtained by the fitting algorithm are presented in Fig. 9.

![Fig. 8. Molar absorption coefficients $\varepsilon$ of eumelanin, pheomelanin, HbO$_2$, and Hb [10,11].](image)

Table 3. Fitted Molar Concentrations of the Primary Absorption Components in Human Skin

|          | Eumelanin (moles/liter) | Pheomelanin (moles/liter) | HbO$_2$ (moles/liter) | Hb (moles/liter) |
|----------|-------------------------|----------------------------|-----------------------|-----------------|
| Normal skin | $4.39 \times 10^{-4}$ | $15.7 \times 10^{-4}$ | $3.18 \times 10^{-5}$ | $2.88 \times 10^{-6}$ |
| Skin with hyperpigmentation | $4.80 \times 10^{-4}$ | $33.1 \times 10^{-4}$ | $2.94 \times 10^{-5}$ | $1.82 \times 10^{-6}$ |

![Fig. 9. Fitting curves, which are the sums of the absorption coefficients of pheomelanin, eumelanin, HbO$_2$, and Hb, calculated using the fitted molar concentrations of the primary absorption components in human skin to reproduce the measured absorption coefficients of (a) normal skin and (b) skin with hyperpigmentation.](image)
Ito et al. [28] summarized the reported contents of eumelanin and pheomelanin in human skin, which differ for different studies. This is because the amounts of these melanin depend on many factors, including the measurement method, race and the measured body part. Therefore, in this discussion, we focused on the changes in melanin contents by hyperpigmentation in our measurement results.

First, Fig. 9 demonstrates that all the measured absorption coefficients of all of the types of skin could be reproduced by properly adding up the absorption coefficients of the relevant substances. Furthermore, the details in Table 3 indicate that the change in hemoglobin is very small between normal skin and skin with hyperpigmentation caused by an anticancer agent, while the amount of pheomelanin is increased by about two times and there is not much change in the amount of eumelanin.

5.4 Statistical study on the fitting algorithm for absorption coefficient analysis

In order to conduct a statistical study on the results of the fitting algorithm, the coefficient of determination $R^2$ [29], which shows the goodness-of-fit, was calculated using MATLAB software. The coefficient of determination is the square of coefficient of correlation between the measurement and the fitted result of the absorption coefficients. Coefficient of determination was also calculated from fitting of the measurement result from each subject, and then the average value of the coefficient of determination was calculated. The results of the coefficients of determination with standard deviations for the normal skin and skin with hyperpigmentation are respectively, $0.975 \pm 0.018$ and $0.987 \pm 0.006$. The coefficients of determination show high value, indicating the fitting algorithm of our study can faithfully reproduce measurement results.

In order to examine the validity of the fitting algorithm used here, the coefficients of determination $R^2$ when using only eumelanin, pheomelanin, or HbO$_2$/Hb for the molar concentrations were calculated, shown in Table 4. From the results of Table 4, the coefficients of determination were close to results of the mixture of all four substances and high value when using only eumelanin and pheomelanin for both the normal skin and skin with hyperpigmentation. However, when Welch's $t$ test [30] for calculating $p$-value was used to compare the coefficient of determination $R^2$ in the case where the mixture of four substances instead of individual substances, there was a significant difference ($p < 0.01$). Also, the standard deviation of the mixture of four substances has the smallest value. Therefore, it was shown that it is necessary to use mixture of four substances, which has the highest coefficient of determination $R^2$, for more accurate fitting to the actual measurements.

|                        | Eumelanin | Pheomelanin | HbO$_2$/Hb | Mixture of all four substances |
|------------------------|-----------|-------------|-------------|--------------------------------|
| Normal skin            | $0.932 \pm 0.035$ | $0.902 \pm 0.050$ | $0.685 \pm 0.080$ | $0.975 \pm 0.018$ |
| Skin with hyperpigmentation | $0.966 \pm 0.015$ | $0.953 \pm 0.027$ | $0.615 \pm 0.054$ | $0.987 \pm 0.006$ |

6. Conclusion

In this study, in order to understand the optical characteristics of hyperpigmentation, we measured the optical properties of the skin of Japanese subjects with hyperpigmentation to investigate the origin of the unique appearance of skin with hyperpigmentation. Based on the measurement results, we examined the physical properties to which this effect could be attributed.

The analysis showed that the amount of pheomelanin significantly increases in hyperpigmentation due to the fluorinated pyrimidine anticancer agent. The results also demonstrated that the fluorinated pyrimidine anticancer agent reduce the scattering coefficient...
of the skin. There is a possibility that the thickness of the surface layer of skin having a high scattering coefficient is reduced due to the fluorinated pyrimidine anticancer agent, and consequently, the measured scattering coefficient of the skin may decrease. Since we did not measure the thickness of this layer, this hypothesis should be confirmed by further study of conducting depth resolved measurements by optical coherence tomography [31,32] and we believe that could confirm our current modeled results.

Based on our measurements and modelling, it can be said that the decrease in the scattering coefficient lowers the reflectance of the skin, making it darker. This characteristic is considered to be one of the potential reasons for the unique appearance of skin with hyperpigmentation. We would like to point out that the optical characteristics of the different types of pigmented skin obtained in this study are considered to be useful for developing cosmetics to cover the unique appearance of hyperpigmented skin, as well as for addressing hyperpigmentation cytologically.

Acknowledgments

We would like to express our sincere thanks to the participating patients, their families, and the staff of the National Cancer Center Hospital.

Disclosures

The authors declare that there are no conflicts of interest related to this article.

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