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Fluorescent image analysis for evaluating the condition of facial sebaceous follicles

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Abstract

Background/purpose—Conventional methods, such as ‘Sebutape’ and ‘Sebumeter,’ can provide quantitative information on sebum excretion but cannot reflect the condition of sebaceous follicles that can be indirectly evaluated with fluorescent colors of sebum. The images of sebum excretion can be obtained with an ultraviolet-A light that is generally called ‘Wood’s Lamp.’ In this study, we describe fluorescent image analysis methods for the detection of sebum and the color segmentation of sebum to evaluate the condition of sebaceous follicles.

Methods/results—For subject-dependent automatic image analysis, we extracted calibrated image analysis methods that were optimized for digital fluorescent images acquired from our imaging system. The calibrated subjective threshold values for sebum detection were determined by statistically analyzing the number of sebum detected by the automatic threshold value method and the subjective threshold value method ($R = 0.947$, the number of volunteers: 29). In fluorescent color analysis of sebum, the calibrated reference color markers for the red and white colors were extracted with a coefficient of variance of $< 10\%$.

Conclusion—We are confident that such calibrated image analysis methods in combination with our imaging system can provide useful quantitative diagnostic information for sebum-related skin pathologies.

Keywords

sebaceous follicle; fluorescent image; sebum; color segmentation; UVA

A fluorescent image can provide functional information for the diagnosis and treatment of skin pathologies that cannot be observed with other imaging modalities (1–6). Sebum can be easily detected with fluorescent imaging. Sebum is a complex oil on the skin that consists of glycerides, free fatty acids, wax esters, squalene, and cholesterol esters, which are secreted from sebaceous follicles (7). Sebum excretion is controlled by sex hormones (androgens) and protects the skin from inflammation, is waterproof, and contributes to body odor. A well-defined sensitive and quantitative evaluation of sebum excretion is useful in evaluating the efficacy of medications designed for the treatment of acne and other skin pathologies (8,9).

Since the first attempt to measure sebum excretion, various methods have been developed (10–12). The most widely preferred methods are ‘Sebutape,’ ‘Sebumeter,’ and ‘Wood’s Lamp.’ The ‘Sebutape’ and ‘Sebumeter’ provide quantitative information for sebum excretion but do not reflect the condition of sebaceous follicles. ‘Wood’s Lamp’ uses a spectral range of ultraviolet- A (UVA) and induces fluorescence of skin chromophores. Conventionally, the fluorescence image has been subjectively evaluated. Recently, the conventional ‘Wood’s Lamp’ has been combined with a high-resolution digital color camera (3,6,13–15), which now makes it possible to analyze fluorescing biological components (e.g. sebum) quantitatively and systematically.

The condition of sebaceous follicles can be indirectly evaluated by analyzing sebum colors with a UVA-induced fluorescent image. For example, sebaceous follicles filled with inflamed sebum emit a brilliant white, with excessive amount of sebum emit red, and with pH unbalanced sebum emit yellow fluorescence (1,2). Usually, normal sebaceous follicles do not fluoresce.

In a previous study (15), we developed a UVA-induced digital facial fluorescent imaging system and proved its clinical efficacy. In this study, we describe image analysis methods in detail to determine the condition of sebaceous follicles by analyzing the fluorescent color information of sebum. A number of facial fluorescent images were acquired, and a color segmentation method was applied to extract useful information on sebum excretion.

Materials and Methods

Facial fluorescent imaging system

The imaging system (Fig. 1) was described in detail in our previous study (15). The imaging box and dark curtain provide a quasi-dark environment, and a digital color camera (Coolpix 8400, Nikon; Tokyo, Japan) was manually set to obtain an optimal facial fluorescent image induced by four UVA lamps. A head-positioning device was integrated into the imaging system to minimize motion artifact during image acquisition.

Image analysis method

Fluorescent images were analyzed with a laboratory-built ‘MatLab’ code. The pattern of sebum excretion was automatically detected by the following procedures. The fluorescent image was complemented and converted into a binary image by automatic threshold value (ATV) determined by Otus’ method (16). The binary image represents the sebum and background area as 1 and 0, respectively. The distribution of sebum is detected with a morphological structuring element in which the results can be varied depending on the selected radius (in this case, the radius of the disk shape was 6). Using the morphological structuring element smooths and fills in or removes the sebum in the binary image. Finally, sebum is optimally changed to be extracted.

In addition to using ATV in sebum detection, a subjective threshold value (STV) determined by an operator was also applied to the first step of the image analysis procedure. The STV for sebum detection was determined for each fluorescent image by qualitatively comparing the fluorescent and the sebum-detected images. The capability of both threshold values for sebum detection was evaluated by computing the percent difference of the number of sebum spots detected by ATV and STV.

$$\text{Percent difference} = \left[\frac{(\text{STV} - \text{ATV})}{\text{STV}} \right] \times 100 \quad (1)$$

In order to compute the sebum-related parameters (area and percent density of sebum spots, mean area and diameter of a sebum spot, and the number of sebum spots) quantitatively, calibration of a physical area was first performed with a fluorescing patch ($1\text{cm}^2 = 510,816$ pixels) by counting the number of pixels in the patch area. The number of sebum spots was counted by detecting and labeling the edges of sebum. The mean area of sebum spots was simply computed by rationing the sebum area to the number of sebum spots. Assuming that sebum spots have a circular pattern, we computed the mean diameter of the sebum spots. The image analysis methods are summarized in Table 1.

Color segmentation (17) was performed to classify the condition of sebaceous follicles because sebum fluoresces different colors depending on the condition of the sebaceous follicles. The background colors of a fluorescent image that have a blue or purple tint might cause errors in the color segmentation of sebum. Therefore, the background colors were removed, maintaining the colors of sebum. In this study, we only considered sebum fluorescing red or white color because of the limitation in recruiting volunteers with various conditions of sebaceous follicles. A representative red- or white-colored sebum was subjectively selected as a reference color marker (RCM) and its own mean red (R), green (G), and blue (B) values were computed. The sebum colors were classified by computing Euclidean Distance (ED) as follows:

$$ED = \sqrt{(R - R_r)^2 + (G - G_r)^2 + (B - B_r)^2} \quad (2)$$

where R , G , and B [R_r , G_r , and B_r] are the values of red, green, and blue [subjective RCM (SRCM)] of sebum, respectively. The smallest value of ED indicates that the pixel is well matched with the SRCM. For example, if an arbitrary pixel has the smallest value of ED, with the SRCM representing the white color, then the pixel is labeled as a white pixel. Finally, the classified fluorescent colors of sebum are displayed with pseudo-colors for better contrast.

Evaluation of image analysis method

The efficacy of the image analysis method was evaluated with 29 fluorescent images obtained from each volunteer. A non-fluorescing patch with an open area (1cm^2) in the center was attached on the mid-forehead of each volunteer for identical area extraction.

For clinical practice, use of the STV for sebum detection and the SRCM for color segmentation is a time-consuming, cumbersome procedure. Therefore, for automatic user-friendly image analysis, we statistically extracted the calibrated subjective threshold value (CSTV) for sebum detection and the calibrated subjective reference color marker (CSRCM) for color segmentation.

By comparing the number of sebum spots detected with both threshold methods, we performed a statistical analysis between the STVs and the ATVs in order to extract subject-dependent optimal CSTV, minimizing the detection error of sebum spots.

The CSRCMs were computed by averaging the RGB values of SRCMs of sebum spots representing the red and white colors from 10 volunteers' fluorescent images. Five sebum spots representing red and white colors, respectively, were subjectively selected from each image, and totally, 50 sebum spots representing each color were statistically analyzed and used to compute CSRCMs.

Results

Figure 2 shows the fluorescent facial images of two volunteers. The black open area (1cm²) on the right picture is due to a non-fluorescent patch. In both figures, the distribution of red and white sebum can be clearly observed.

Figure 3 shows a graphic–user interface program to quantitatively compute the sebum-related parameters listed in Table 1 and analyze sebum colors from acquired facial fluorescent images. In addition, it qualitatively shows the pattern of sebum distribution. An example of the quantitative analysis of sebum-related parameters is listed in Table 2.

Figure 4 shows the percent difference of the number of sebum spots detected by ATV and STV. In the analysis, the STV was used as a reference for comparison because it shows similar sebum detection capability when compared with visual inspection by an observer. As illustrated in the figure, the STV resulted in better sebum detection capability compared with ATV. In all cases, the STV detected much greater numbers of sebum spots that were not detected by the ATV. On average, the sebum detection capability of STV was 46% higher than that of the ATV.

Figure 5 shows a linear regression result between the STV and ATV, which has a strong positive correlation coefficient ($R = 0.947$, $P < 0.01$). From the linear regression, an equation ($CSTV = 0.934ATV + 0.082$) for CSTV was extracted to determine automatically subject-dependent STV for sebum detection.

The sebum colors were classified as red or white. The procedure of color segmentation is illustrated in Fig. 6: (a) selection of a region of interest; (b) conversion into a binary image and detection of sebum; (c) removal of background colors except for sebum colors; (d) segmentation of red-colored sebum; (e) segmentation of white-colored sebum; and (f) pseudo-coloring of red and white sebum for contrast enhancement.

The CSRCM for red and white color was determined by computing its own mean RGB values from 50 sebum spots, respectively. The mean RGB values of red (white) color were 168 (154), 128 (153), and 230 (223), respectively. The statistical result is summarized in Table 3. Using the mean RGB value for each color, the calibrated ED (CED) for color segmentation of each color was described as follows:

$$CED = \sqrt{(R - R_c)^2 + (G - G_c)^2 + (B - B_c)^2} \quad (3)$$

where R , G , and B (R_c , G_c , and B_c) indicate the mean values of red, green, and blue color of sebum (CSRCM), respectively.

Discussion

The quantitative information on sebum excretion listed in Table 2 might provide useful information for the diagnosis/therapy of sebum-related pathologies (for instance, acne). In addition to the analysis of sebum, fluorescent imaging can be successfully utilized for quantitative analysis of other skin conditions, such as keratosis, carcinoma, acne, sun spots, hyper- and hypo-pigmentation, and moles. Figure 2a shows an example of the fluorescent image of a volunteer with hypo-pigmented skin (vitiligo) around the mouth.

The use of an optimal threshold value for converting a fluorescent image into a binary image plays an important role in the determination of sebum detection capability. In the analysis of

various fluorescent images, we realized that using ATV compared with STV might miss many sebum spots. In order to enhance sebum detection capability in an automatic fashion, the capability of ATV was statistically compared with that of STV. ATV presented poor sebum detection capability compared with STV, resulting in a mean percent difference of 46% in the 29 volunteers study (Fig. 4). The number of sebum spots detected using STV was much more successful than using ATV among all volunteers. Such a huge difference might be caused by different image quality among the fluorescent images. It might be difficult to selectively detect sebum spots when: (1) the background skin has a non-homogeneous distribution of pigmentation; (2) the images are blurred due to the motion artifact of volunteers during image acquisition; (3) the fluorescent intensity of sebum is not strong enough compared with the background skin color; or (4) the size of the sebum spots is not large enough to be detected. Sebum under such conditions might not be detected by the ATV algorithm but may be observed by the operator's visual inspection. In fact, many sebum observed by an operator were not detected by ATV. As a result, STV that presented a sebum detection capability comparable to operator-dependent visual inspection is strongly recommended for subject-dependent optimal sebum detection.

For standard clinical practice, automatic online analysis of sebum excretion has to be relatively fast and produce reliable and reproducible results. Therefore, the use of STV is not appropriate for practical clinical application. In order to solve the limitation of STV, we extracted a CSTV by statistically analyzing the number of sebum spots detected by ATV and STV (Fig. 5). Using the extracted CSTV, sebum spots can be automatically detected with a detection capability that is comparable to STV.

The color segmentation method is useful for differentiating sebum colors. An example is summarized in Table 2 that shows quantitative information of color-segmented sebum. Subjective selection of RCM is tedious work and might cause operator-dependent errors in sebum color analysis. CSRCM can be used for automatic sebum color segmentation. The coefficient of variation was <10% for the RGB values of each color (red and white). In fact, we confirmed that CSRCM successfully separated sebum colors in multiple validated fluorescent images. However, it should be remembered that the CSRCM are device-dependent. Therefore, CSRCM are only effective for the fluorescent images obtained with our imaging system that has a constant image acquisition setup.

Sebum can fluoresce more colors depending on the condition of sebaceous follicles as listed in Table 4 (1,2). However, in this study, we observed only two fluorescent colors (red and white) due to the limitation of available volunteers. Therefore, only two colors were considered for the color segmentation. However, this method can be further utilized to differentiate more fluorescent colors of sebum.

Currently, the classification of fluorescent colors of sebum is based on a broadband, three-color (red, green, and blue) images. Therefore, the color of sebum might be subjectively described depending on the operator and might vary depending on the color camera used. Such issues might be solved using a multi-spectral imaging system in which fluorescent images can be taken at narrowband wavelengths. However, motion artifact during the image acquisition has to be seriously considered in color segmentation. As a result, there is a trade-off between broadband color imaging and multi-spectral imaging in color segmentation of sebum. In a future study, such issues will be evaluated.

Conclusion

In conclusion, a fluorescent image of sebum excretion was processed and analyzed to extract quantitative information on sebum-related parameters. For routine clinical use, existing image

processing algorithms were calibrated to optimize sebum detection. The calibrated sebum detection method resulted in a good correlation with the existing method. The fluorescent colors of sebum were successfully separated with the calibrated color segmentation method. We are confident that the image analysis methods combined with our imaging system can provide useful diagnostic information for skin pathologies related to sebum. In order to ensure the clinical efficacy of the image analysis methods, we are performing a clinical study on acne patients.

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Fig.1. Facial fluorescent imaging system that consists of a digital color camera, four ultraviolet-A lamps, and a head-positioning device integrated into an imaging box.

Fig.2.
Facial fluorescent images of two volunteers acquired with the imaging system.

Fig.3. Graphic–user interface program coded with MatLab that quantitatively analyzes sebum-related parameters from a fluorescent image.

Fig.4.

Percent difference $[(STV - ATV)/STV \times 100]$ of counted sebum spots using a subjective threshold value (STV) and an automatic threshold value (ATV) to detect sebum. In the computation, STV was used as a reference. The average percent difference was 46% among all volunteers.

Fig.5. Linear regression between the automatic threshold value (ATV) and subjective threshold value (STV) in determination of threshold values for sebum detection ($CSTV = 0.888ATV + 0.109$, $R = 0.96$). Twenty-nine data points from 29 volunteers are involved in the statistical analysis. CSTV, calibrated subjective threshold values.

Fig.6.

Color segmentation procedure of sebum: (a) selection of a region of interest; (b) conversion into a binary image and detection of sebum; (c) removal of background colors except for sebum colors; (d) segmentation of red-colored sebum; (e) segmentation of white-colored sebum; and (f) pseudo-coloring of red and white sebum for contrast enhancement.

TABLE 1

Image analysis methods to extract quantitatively sebum-related parameters from fluorescent images

Parameters	Computation methods
Total pixel number	Pixel counting (image size)
Total area (cm ²)	Total number of pixels/10,816
Sebum area (cm ²)	Total pixel number of sebum/10,816
Percent density of sebum (%)	(Sebum area/total area) × 100
Number of sebum spots	By edge detecting and labeling
Mean area of a sebum spot (cm ²)	Sebum area/number of sebum spots
Mean diameter of a sebum spot (cm)	$2 \times (\text{mean area}/\pi)^{0.5}$

TABLE 2

An example of sebum-related parameters extracted by using image analysis method listed in Table 1

Parameters	Red color sebum	White color sebum	Total sebum
Pixel number	40,800	40,800	40,800
Total area (cm ²)	3.77	3.77	3.77
Sebum area (cm ²)	0.08	0.024	0.104
Percent density (%)	2.12	0.64	2.76
Number of sebum	103	72	175
Mean area (cm ²)	7.77×10^{-4}	3.33×10^{-4}	5.94×10^{-4}
Mean diameter (cm)	0.0315	0.0207	0.0302

TABLE 3
Statistical analysis of reference color marker (RCM) computed from 50 sebum spots

Fluorescent color RGB color	Red color			White color		
	Red	Green	Blue	Red	Green	Blue
Mean	168	128	230	154	153	223
SD	9.23	6.13	15.54	11.61	10.12	7.75
CV (%)	5.5	4.78	6.76	7.56	6.63	3.47

TABLE 4

Various fluorescent colors that might be observed depending on the condition of sebaceous follicles

Condition of sebaceous follicles	Fluorescent color
Inflamed not open	White
Excessive amount of sebum	Red
Not open with altered pH-bacteriostatic	Yellow
Acne	Brown