

INVESTIGATIVE REPORT

In vivo Estimation of Stratum Corneum Thickness from Water Concentration Profiles Obtained with Raman Spectroscopy

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Stratum corneum thickness was estimated from water concentration profiles of the skin measured by a confocal Raman spectrometer. Stratum corneum apparent thickness (SCAT) was defined as the depth where the water content reached an almost constant value. Site variations were determined using 15 healthy Japanese subjects (6 males, 9 females), and age variations at the cheek and forearm were examined using 27 female Japanese subjects. There were marked site variations in mean SCAT; 16.8 μm for cheek, 22.6 μm for volar forearm, 29.3 μm for back of the hand, and 173.0 μm for palm. These variations were similar to reported stratum corneum thickness values obtained in biopsy tissues. The SCAT tended to become age-dependently thicker at the forearm, but not at the cheek. In addition, SCAT was increased up to two-fold by hydration for 90 min, while lesser increases were seen with shorter hydration periods. **Key words:** *in vivo*; Raman; stratum corneum; thickness; water concentration profile.

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The skin comprises two main layers: dermis and epidermis, the latter consisting of basal layer, stratum spinosum, stratum granulosum, and stratum corneum (SC). Regarding SC thickness, chronological and photo-ageing changes (1, 2), the relationships between epidermal thickness, pigmentation and human photosensitivity (3), and regional differences (4, 5) have been reported. Measurement of SC thickness is usually based on light microscopy. However, the conventional formalin-paraffin process used in the light microscopy method changes the SC thickness. Recently, a freezing technique (6, 7), was introduced to minimize changes in SC thickness.

There have been several attempts to develop non-invasive techniques to measure SC thickness using high-frequency ultrasonography (8), high-frequency magnetic resonance imaging (9), and pulsed terahertz radiation (10). However, these techniques do not have sufficient resolution to measure SC other than at the heel or palm, and they cannot provide the same resolution and precision as the standard light microscopy method.

Using *in vitro* methods, it has been reported that the water content gradually increases, going from the upper part of SC down to the viable epidermis, reaching an almost constant value thereafter (11, 12). Warner et al. (11) using *in vitro* X-ray microanalysis, reported a continuous increase in the water content of SC (g water/g tissue), from about 15% to 25% at the skin surface to a constant level of about 70% in the viable stratum granulosum, which corroborates the theoretical prediction of water concentration profile across the skin (13). The same authors also showed that a large discontinuity in water content occurred at the boundary between the SC and stratum granulosum, which makes it possible to estimate SC thickness *in vivo* by detection of this interface.

Regarding non-invasive methods for monitoring water content in the skin *in vivo*, instruments based on electrical properties, such as conductance (14) and capacitance (15, 16), and spectroscopic techniques, such as near-infrared spectroscopy (17–20), have been used. However, it is difficult to control measurement depth using these instruments. Recently, a confocal Raman spectrometer was used *in vivo* to measure molecular concentration-depth profiles in the skin for water and amino acids, with a 2 μm interval (21–23).

The aim of this study was to estimate human SC apparent thickness (SCAT) by measurement of *in vivo* water concentration profiles using Raman spectroscopy, and in addition to determine site and age variations of SC thickness.

MATERIALS AND METHODS

Instrumentation

Raman spectra were obtained at different depths below the skin surface using a confocal Raman spectrometer (Model 3510, River Diagnostics BV, Rotterdam, The Netherlands), which is designed for *in vivo* investigation of human skin (21–23). The skin was positioned on an aluminium stage containing a CaF_2 window, which served as a reference plate to determine skin surface position and to prevent movement artefacts.

Subjects

A total of 33 healthy Japanese volunteers, 6 men (age range 34–54 years, mean 43.2 years), and 27 women (age range 23–76 years, mean 56.3 years) were enrolled. Among them, site variation experiments were conducted at five sites, i.e. cheek, upper arm (flexor aspect), volar forearm, back of the hand, and palm (ball of the thumb), of 15 subjects, (all the men and 9 women age range 23–49 years, mean 35.4 years). For

determination of age variations, Raman spectra obtained at the cheek and volar forearm of all the female subjects were used. None of them had any history of skin disorders and none was taking medication at the time of the experiment.

The procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation in our company (Shiseido Co. Ltd) and with the Helsinki Declaration.

In vivo Raman spectra

Subjects washed the measurement areas once with solid soap 1 h before Raman measurement. *In vivo* depth profiles of Raman spectra were measured at 2 μm intervals from the skin surface towards the interior, along a line perpendicular to the skin. Raman spectra were recorded with an excitation wavelength of 671 nm and a measurement time of 1 second per spectrum in the 2600–4000 cm^{-1} region. Spectra were calibrated and corrected for the instrument response (20–22). The laser power on the skin was 17.0–19.0 mW. All measurements were performed at 23.5–24.5°C and 57–65% relative humidity.

Hydration experiment

The hydration experiment was carried out on the volar forearm of one female volunteer (aged 35 years). The skin was hydrated for 15, 50 and 90 min by application of 120 μl distilled water on a 6 \times 6 mm cotton wool patch, covered with an adhesive bandage. Raman spectra of the skin were recorded before and after hydration.

Calculation of water content

Water-to-protein ratio in the SC was calculated as the ratio between the intensity of the Raman signal of water (due to OH-stretching vibrations) integrated over 3350–3550 cm^{-1} and that of protein (due to CH₃-stretching vibrations) integrated over 2910–2966 cm^{-1} . Water content (mass-%), expressed in grams of water per 100 g of wet tissue (water + dry mass), was calculated from the water-to-protein ratio (21–23).

Estimation of stratum corneum thickness

SC thickness was estimated from the water concentration profile (Fig. 1). The first derivative of water concentration profile was

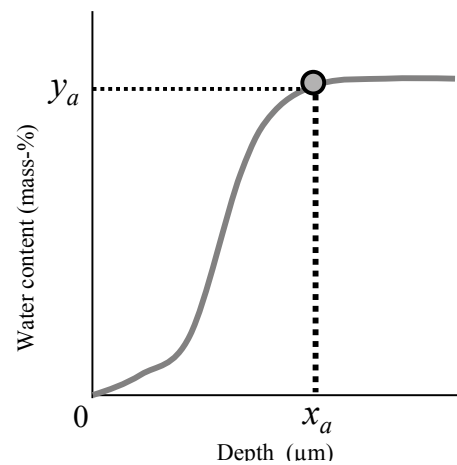


Fig. 1. The principal for estimation of stratum corneum apparent thickness (SCAT) from water concentration profile. SCAT was defined as x_a (μm), i.e. the depth at which the water content reached an almost constant value.

calculated by moving average to find the depth (x_a) at which the rate of change became almost zero, i.e. where the water content reached an almost constant value (y_a). We defined SCAT as x_a (μm).

Data analysis

MATLAB (Version 7.0.4 (R14), The MathWorks, Inc., Natick, USA) and Microsoft Excel 2002 (Microsoft Corporation, WA, USA) were employed for data analysis. Analysis of variance was used to compare differences between body sites. A p -value < 0.05 was considered significant.

RESULTS

Site variations in water concentration profiles

Using Raman spectrometry the water content was measured in the skin of 15 healthy subjects. Fig. 2 shows the *in vivo* water concentration profiles of the cheek,

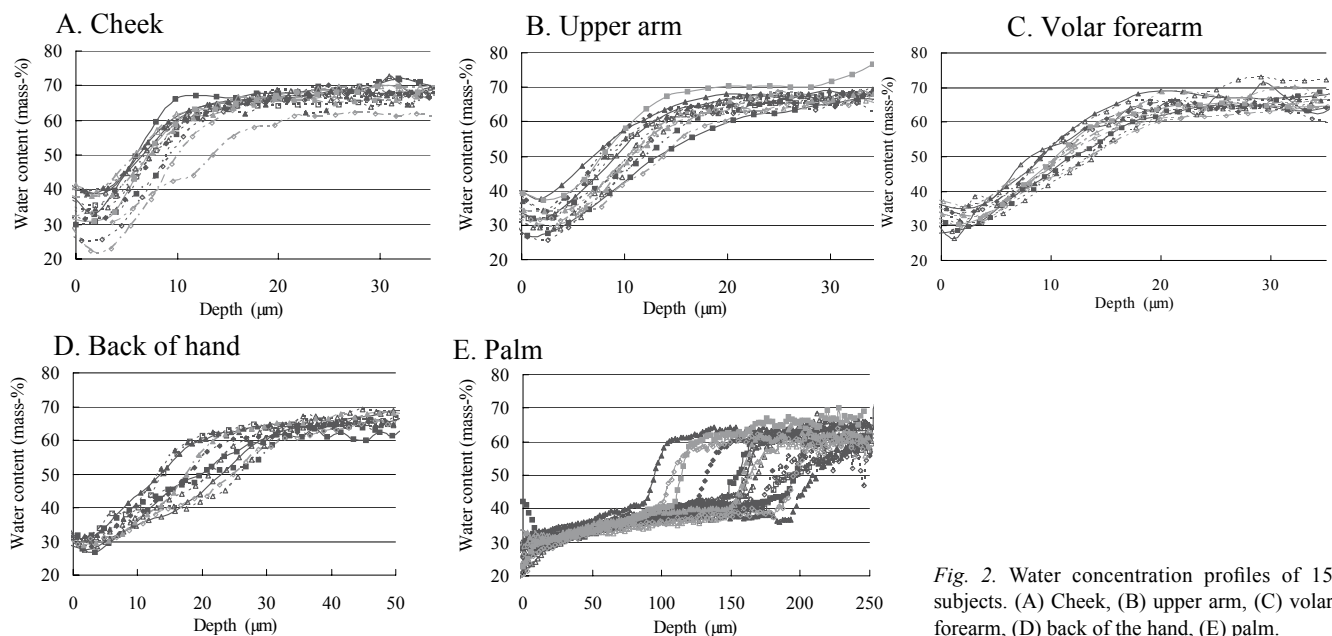


Fig. 2. Water concentration profiles of 15 subjects. (A) Cheek, (B) upper arm, (C) volar forearm, (D) back of the hand, (E) palm.

upper arm, volar forearm, back of the hand and palm, respectively. The water concentration profiles of the cheek, upper arm, volar forearm and back of the hand were similar: the water content at the skin surface was 30–40%, and gradually increased with increasing depth until it reached a constant value of 65–70%. In contrast, the water content of the palm was around 20–30% at the skin surface, then slightly increased to 40%, and finally rapidly increased to a constant value of 60–70%. Individual variations were seen both in water content at the skin surface and in constant water content value in deeper parts, especially at the palm.

Site variations in stratum corneum apparent thickness

SCAT was calculated as described in the Materials and Methods section. Examples of water concentration profiles from cheek and palm are shown in Fig. 3A. The first derivative of the water concentration profiles are shown in Fig. 3B and C. SCAT was defined as the depth at which the first derivative fell below 0.5 (for cheek, upper arm, volar forearm and back of hand) or 0.1 (for palm) and the water content value became almost constant (shown with an arrow in Fig. 3B and C).

Site variations of SCAT were compared (Table I): mean SCAT was lowest at the cheek (16.8 μm), intermediate at the upper arm and volar forearm, slightly thicker at the back of the hand (29.3 μm), and thickest at the palm (173.0 μm). By analysis of variance there were significant differences ($p < 0.001$) between the palm and cheek, palm and upper arm, palm and forearm, and palm and back of the hand.

Age variations in stratum corneum apparent thickness

There was a positive correlation between age and SCAT measured at the forearm, while no correlation was found

Table I. Stratum corneum apparent thickness (SCAT) at different body sites

	No. of subjects	Mean	Standard deviation
Cheek	15	16.8	2.84
Upper arm	15	21.8	3.63
Forearm	14	22.6	4.33
Back of hand	13	29.3	6.84
Palm	15	173.0	36.96

Significant differences ($p < 0.001$) were seen between the palm and cheek, palm and upper arm, palm and forearm, and palm and back of hand.

at the cheek in female subjects. Age variations in SCAT at the forearm were larger than those at the cheek (Fig. 4).

Change in stratum corneum apparent thickness after hydration

It is clear that the water content value becomes higher in the upper area of the SC after hydration when studying the water concentration profiles of the skin before and after hydration for 50 min in one healthy women (Fig. 5). By contrast, the water content value deeper in the skin (around 65%) was unchanged by the 50 min hydration process. Table II shows the change in SCAT at the volar forearm after hydration for 15, 50 and 90 min with water-soaked cotton wool. SCAT was increased almost two-fold from the initial value after hydration for 90 min, while smaller increases were seen at short hydration periods.

DISCUSSION

This study examined non-invasive estimation of SC thickness by calculation from the water concentration profiles obtained with a confocal Raman spectrometer (21–23). From previous theoretical prediction (13) and *in vitro* X-ray analysis (11) it was suggested that water

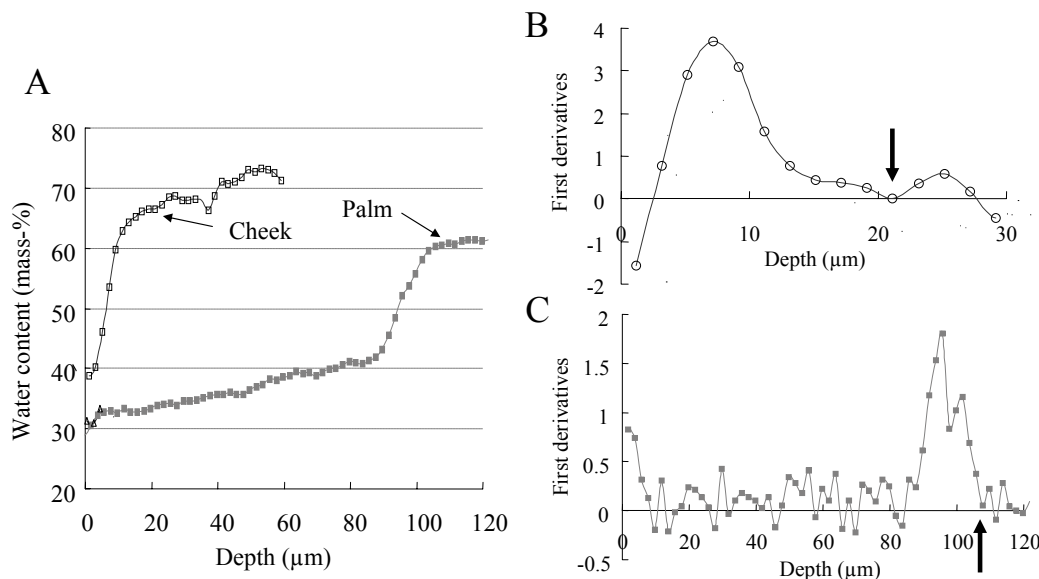


Fig. 3. (A) Water concentration profile and (B, C) first derivative profiles of the cheek and palm, respectively. Stratum corneum apparent thickness (SCAT) is defined as the depth at the position indicated with arrows. Examples from one individual.

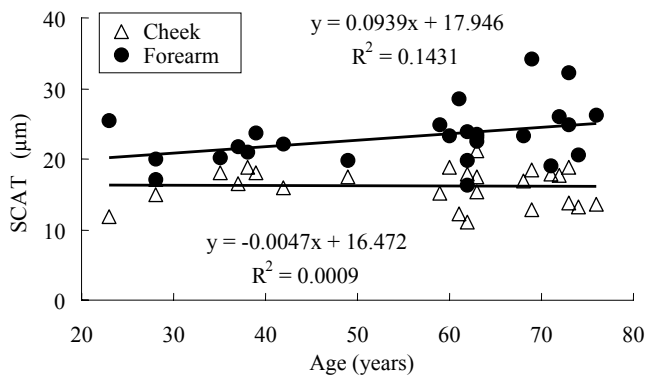


Fig. 4. Age variations in stratum corneum apparent thickness (SCAT) at cheek and forearm. Regression lines and equations are shown for cheek (top) and forearm (bottom) separately.

content value reached an almost constant value at viable granulosum. However, as it is difficult to calculate the boundary between SC and stratum granulosum from water concentration profiles, we defined SCAT as the depth (x_a) where the water content reached an almost constant value. Therefore, SCAT would include the upper part of the stratum granulosum in addition to the SC. As with previously reported *in vivo* methods for calculating SC thickness (8–10), it is not clear whether they accurately detect the boundary between SC and stratum granulosum.

The SC thickness at the volar forearm of Nordic subjects measured by *in vitro* light microscopy that does not include formalin-paraffin processing was 18.3 µm (6, 7). Analogously the SC thickness at the palm, measured with *in vivo* pulsed terahertz radiation, was 170 µm in Caucasian subjects (10). The corresponding SCAT values, calculated from the water concentration profile of Japanese subjects, were 22.6 µm at the volar forearm and 173 µm at the palm, i.e. almost identical to previous estimations of SC thickness.

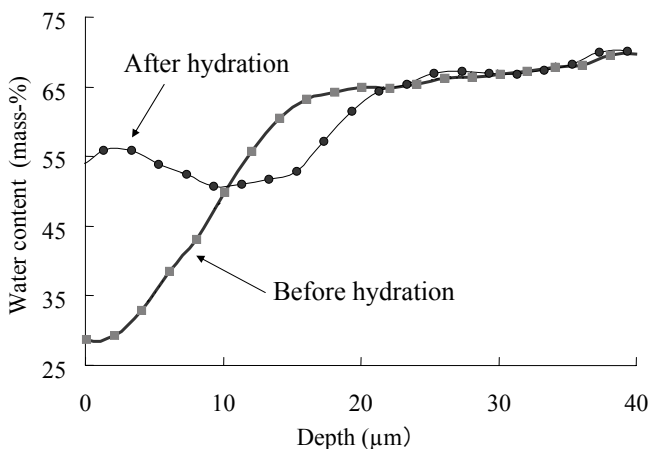


Fig. 5. Water concentration profiles of forearm skin before and after hydration for 50 min with water-soaked cotton wool. Stratum corneum apparent thickness was 18.1 µm before hydration and 25.3 µm after hydration.

Table II. Change in stratum corneum apparent thickness (SCAT) at volar forearm after hydration for 15, 50, and 90 min with water-soaked cotton wool

Hydration period (min)	Initial (µm)	After hydration (µm)	Ratio*
15	18.4	19.1	1.04
50	18.1	25.3	1.40
90	18.7	36.6	1.95

*Ratio of SCAT after hydration against initial SCAT.

Regarding site variations, the mean SC thickness determined by standard light microscopy (24), varied from being high at the palm (90 µm) and back of hand (65 µm), to low at the cheek (15 µm) with intermediate values observed at volar forearm (45 µm) and upper arm (57 µm). However, as the authors used the formalin-paraffin technique, which influences SC thickness, absolute values are not comparable with our method. Ya-Xian et al. (25) reported that the number of cell layers of the SC was 10 at the cheek, 14 at the upper arm, 16 at the volar forearm, 25 at the back of the hand and 50 at the palm. This site variation is consistent with our data, on the assumption that SC thickness is correlated with the number of cell layers of the SC. Sandby-Möller et al. (7) demonstrated that the influence of body site far exceeds the inter-individual differences. Our data (Table I) support these findings.

As for change with ageing, Sandby-Möller et al. (7) reported that SC thickness at the forearm was independent of age; their age range was 20–68 years (Nordic males and females). Our data showed that SCAT was especially high in the oldest Japanese female subjects (68–76 years). Perhaps if previous data had included more elderly subjects, the results might have looked different. Also, gender differences should be considered. Ya-Xian et al. (25) found an age-dependent increase (1–97 years) in the number of cell layers of the SC at the cheek in Japanese male subjects, but not in females. We too found a small age variation in SCAT at the cheek in females (Fig. 5).

Recently, Huzaira et al. (26) reported SC thickness estimated from horizontal images of the skin by *in vivo* reflectance confocal microscopy, obtaining mean values of 9.6 µm at the volar forearm and 12.1 µm at the cheek, which are much lower than ours and those of previous investigators using the new light microscopy method (6, 7). In addition, site variations in the report by Huzaira et al. (26) were different from ours and previous report (24).

Finally, we followed the change in SCAT caused by a hydration process (Fig. 5). It was previously reported that the average thickness of a corneocyte at a low hydration level (18–26% wt/wt) is 300 nm, and this was increased up to two-fold at hydration levels of 57–87% wt/wt, as evaluated with cryo-scanning electron microscopy (27). Our *in vivo* finding, that the SCAT after hydration for 90 min was increased 1.95

times from the initial value (Table II), supports previous *in vitro* results.

As changes in SCAT appear to mimic SC thickness measured by other methods, the SCAT value promises to be useful for recording changes in SC thickness *in vivo*.

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