Supplementary file

Non-invasive characterization of the relationship between skin microrelief and dermal–epidermal junction topography using line-field confocal optical coherence tomography (LC-OCT)

Ken Uda[1 *], Kazuchika Iwami[2], Naoko Yoshida[2], Toshiyuki Homma[1 *]

Supplementary Information

Analysis of parameters other than DEJ undulation

Although this study primarily verified correlations using LC-OCT acquisition data (different parameters pertaining to the internal structure described above), analyses were also conducted as baseline data to examine the correlations between volunteer age and sex. The analysis results and considerations are summarized below and presented in the Supplementary Information.

This supplementary material has been included by the authors to provide readers with additional information on the results.

The components of this Supplementary Material document are as follows:

Figure S1 Analysis of the effects of age and sex on stratum corneum hydration in the forearm skin.

Figure S2 Quantification methods for sulci cutis and data showing a decrease in line density with age.

Figure S3 Correlation with stratum corneum cell area and multilayering rate with age.

Figure S4 Correlation between ceramide subclass types in the stratum corneum.

Table S1 Summary of volunteer information and results for this study.

Table S2 Correlation coefficients between stratum corneum hydration with age for different parameters.

Figure S1 Correlation with stratum corneum hydration.

This study not only presents average values for three arm locations but also confirms differences in stratum corneum hydration across different upper arm regions among volunteers. The results showed no significant correlation between the hydration and age, although a tendency toward increased hydration was observed with aging (**a**). Although differences between sites were minimal on the inner forearm alone, the hydration increased from Arm3 to Arm1 (distal forearm). Based on these results, averages for the three sites were calculated. Additionally, men showed a significantly higher stratum corneum hydration than women (**b**).

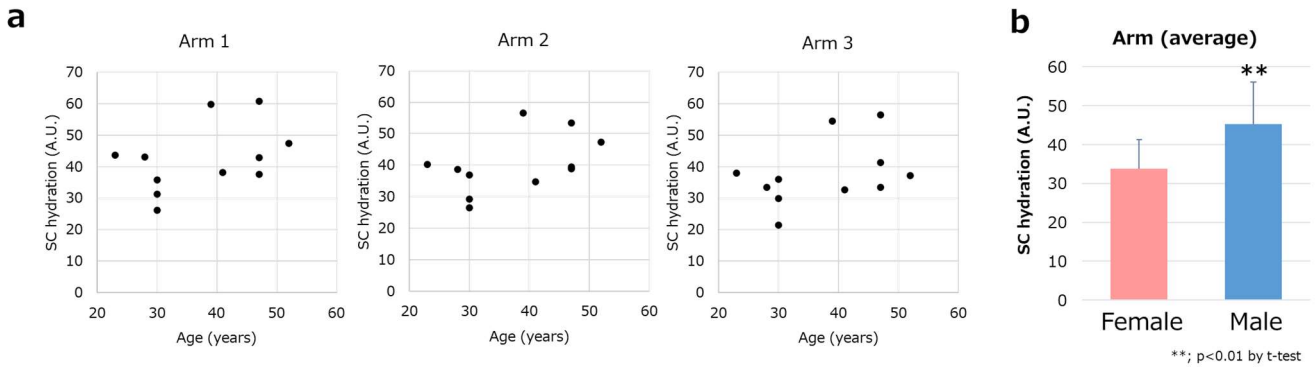

Figure S1 Stratum corneum hydration measurement results.

(**a**) The horizontal axis represents age, and the vertical axis represents the SC hydration for each volunteer, with data from the arm region shown. (**b**) The average SC hydration for each sex (5 females, 6 males). No significant difference in age distribution was observed between male and female volunteers (data not shown).

Figure S2 Method for extracting microrelief quantitative values and correlation with age.

The method for extracting microrelief parameters from dermoscope images of the skin surface is described below. *Sulci cutis* areas are visually labeled (blue) (**a**). The labeled layer was binarized, and 1-pixel-wide line (red) segments were extracted via skeletonization (using the skeletonize function in the ImageJ software). The ratio (%) of the main lines to the measured area was used as the line density of the microrelief. The microrelief line density demonstrated a negative correlation with age, indicating that the microrelief became coarser with aging (**b**).

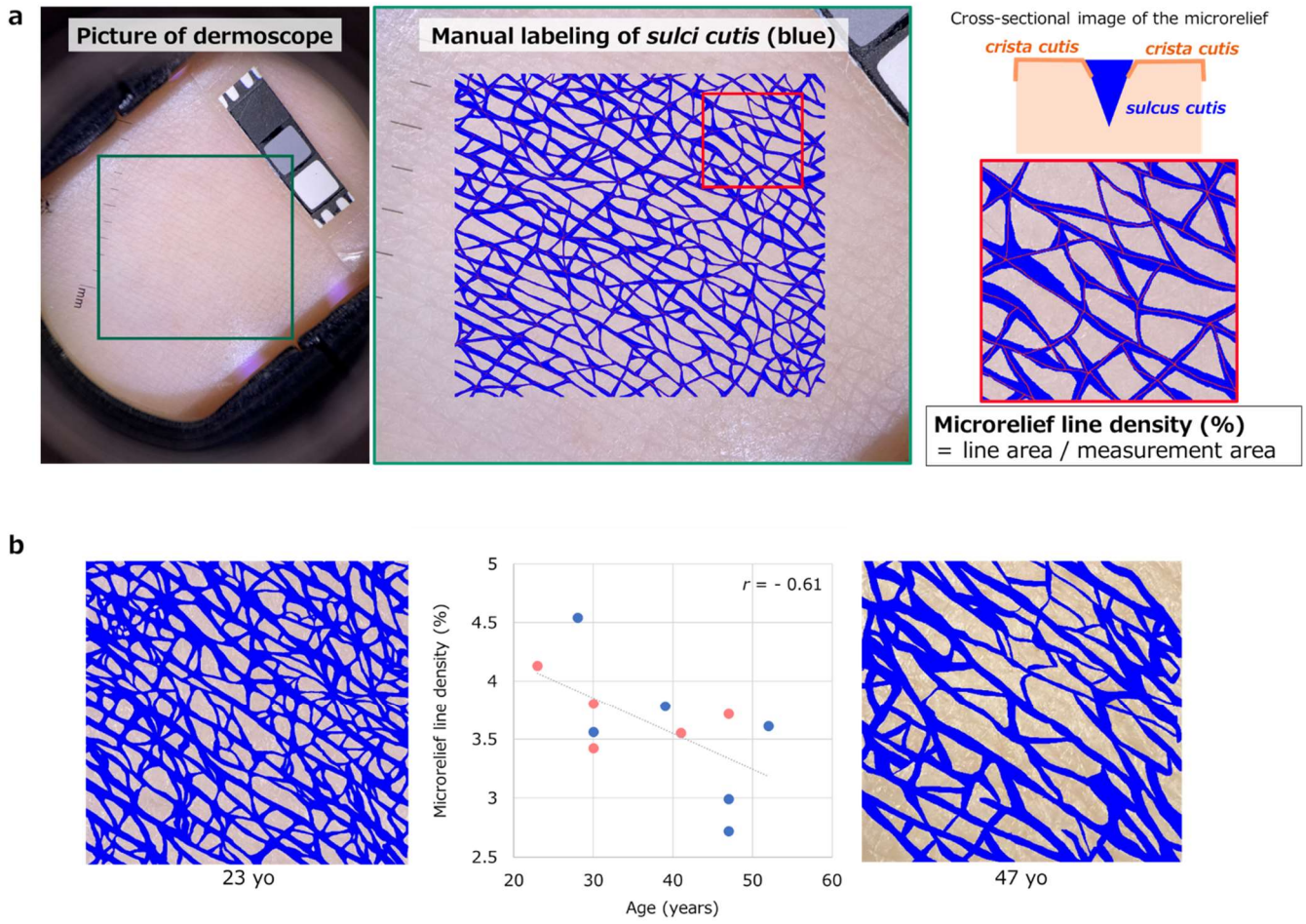

Figure S2 Method for calculating microrelief quantitative values and correlation between microrelief line density with age.

(**a**) Dermoscopy images of the skin surface and methods for measuring microrelief line density. (**b**) Correlation diagram of microrelief line density with age and representative dermoscopy images of young and elderly individuals.

Figure S3 Correlation with stratum corneum cell area and multilayering rate with age.

Similar analyses were performed on stratum corneum cells obtained via tape stripping from the forearms. Transferring the cells to a glass slide and performing stratum corneum staining yielded cell area and multilayering rate values. The results demonstrated no significant difference in stratum corneum cell area (**a**); however, a strong positive correlation with age was observed for stratum corneum multilayering rate, which is used as an indicator of skin condition (**b**). Therefore, we confirmed that the stratum corneum multilayering rate can serve as a good indicator of the effects of aging as a skin parameter.

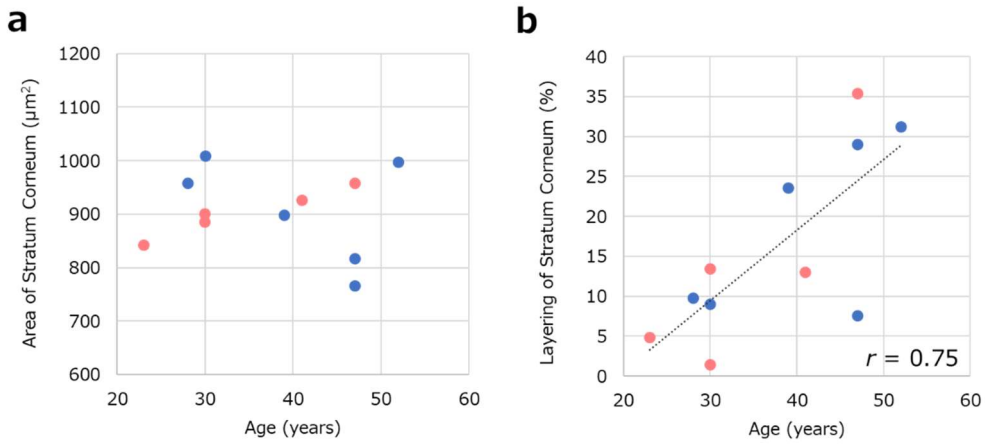

Figure S3 Stratum corneum area and stratification rate results.

Figure S4 Correlation between ceramide types in the stratum corneum and each parameter.

We analyzed different ceramides in the tape-stripped stratum corneum. The composition ratios of each ceramide type closely matched those of the previously reported comprehensive measurements of stratum corneum ceramides. A correlation analysis between measured ceramide types and age or stratum corneum moisture content revealed diverse patterns: some ceramides displayed positive correlations, whereas others showed negative correlations, depending on the ceramide type. Results replicated previously reported correlations, such as those between ceramide EOH and EOS with stratum corneum hydration (Table S2), and the NP/NS ratio with age reported as a potential skin condition marker (**a**). These findings suggest that these ceramide types are useful parameters for indicating deteriorating skin conditions, such as rough skin. Conversely, ceramide AS exhibited statistically significant differences between men and women, a finding not previously reported (**b**). As demonstrated herein, correlations between ceramide types were also observed in the internal structure (DEJ undulation index) as measured by LC-OCT (Fig. 7h). We believe that the composition of stratum corneum ceramides could provide a crucial perspective for verifying new mechanistic hypotheses, as summarized in Tables S1 and S2.

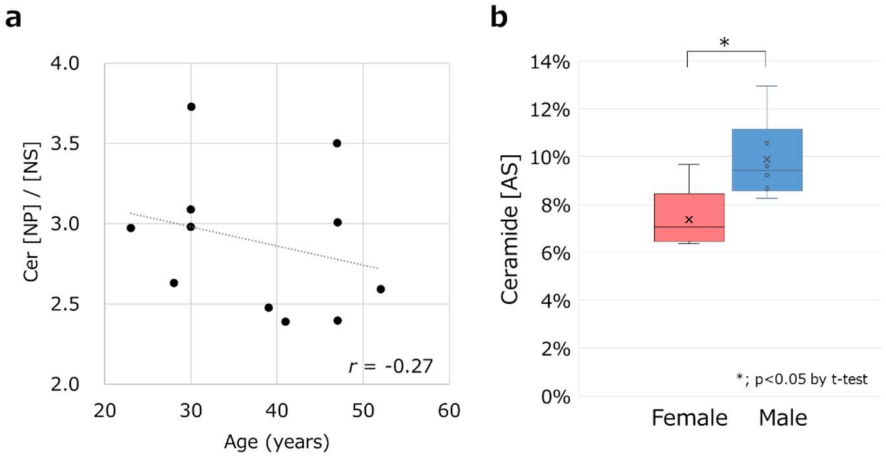

Figure S4 Relationship between ceramide NP/NS ratio and age, and comparison of ceramide AS ratio between men and women.

Table S1 Summary of volunteer information and results for this study.

			Line density	SC area	SC layering	Epidermis	SC hydration	Total ceramides	Ceramide	Ceramide subclass (%)										
Volunteer	Age	Sex	(%)	(μm2)	(%)	(μm)	(A.U.)	(ng/μg)	[NP]/[NS]	NP	ADS	AS	NH	AH	AP	NDS	NS	EOH	EOP	EOS
1	41	F	3.6	925.3	13.0	60.1	35.2	74.9	2.4	41.97	0.51	7.24	1.45	2.03	0.29	15.57	17.56	0.25	5.49	7.64
2	23	F	4.1	842.1	4.8	62.3	40.6	75.1	3.0	43.15	0.29	6.57	1.19	1.02	0.53	12.63	14.51	0.16	15.26	4.67
3	30	M	3.6	1009.0	9.0	58.2	36.3	68.8	3.7	49.76	0.41	8.27	0.95	1.36	0.56	19.81	13.34	0.14	1.04	4.36
4	47	F	3.7	957.7	35.4	52.9	38.5	114.1	3.5	53.94	0.41	6.36	1.20	1.31	0.62	15.39	15.41	0.18	0.30	4.86
5	30	F	3.4	900.1	13.4	54.3	28.5	77.8	3.0	49.34	0.28	7.07	1.34	1.35	0.29	17.66	16.55	0.18	0.76	5.19
6	52	M	3.6	996.5	31.2	57.0	44.0	55.9	2.6	44.99	0.39	10.57	1.45	1.88	0.47	15.42	17.34	0.19	2.60	4.70
7	28	M	4.5	958.8	9.8	55.0	38.4	62.1	2.6	47.27	0.38	9.61	1.39	1.80	0.43	13.46	17.96	0.18	3.50	4.03
8	39	M	3.8	898.4	23.6	60.2	57.0	43.5	2.5	40.08	0.43	12.96	1.76	2.31	0.46	15.32	16.17	0.22	4.52	5.78
9	30	F	3.8	885.4	1.4	62.7	26.4	55.8	3.1	50.96	0.36	9.68	1.56	1.97	0.33	12.59	16.50	0.24	0.97	4.85
10	47	M	2.7	817.6	29.0	59.4	39.3	85.1	2.4	40.81	0.47	9.24	1.48	1.70	0.51	15.71	17.01	0.28	4.21	8.57
11	47	M	3.0	766.2	7.6	60.3	56.9	57.4	3.0	49.88	0.30	8.68	0.93	1.44	0.38	11.66	16.57	0.28	1.07	8.81

Table S2 Correlation coefficients between age and stratum corneum hydration for different parameters.

	Correlation coefficients (r)	
Parameters	Age	SC hydration
Line density	-0.61	-0.17
SC area	-0.06	-0.33
SC layering	0.75	0.25
Epidermal thickness	-0.19	0.17
SC hydration	0.45	-
Total ceramides	0.15	-0.37
Cer [NP]/[NS]	-0.27	-0.25

	Correlation coefficients (r)	
Ceramide subclass	Age	SC hydration
NP	-0.08	-0.34
ADS	0.46	0.03
AS	0.21	0.49
NH	0.07	-0.06
AH	0.28	0.18
AP	0.16	0.26
NDS	-0.01	-0.29
NS	0.36	0.02
EOH	0.52	0.28
EOP	-0.39	0.12
EOS	0.54	0.38