Acta Dermatovener (Stockholm) 59: 285–291, 1979

A QUANTITATIVE METHOD FOR THE ASSESSMENT OF THE MICROTOPOGRAPHY OF HUMAN SKIN

S. Makki,[1,2] J. C. Barbenel[2] and P. Agache[1]

[1]*Clinique Dermatologique Universitaire, Faculté de Médecine et Pharmacie, Besançon Cedex, France and* [2]*Bioengeneering Unit, University of Strathclyde, Glasgow, Scotland*

Abstract. The skin relief influences the exterior aspect of the skin which is very sensitive to aging. It could also be related to the mechanical properties and structure of both dermis and stratum corneum. Consequently, quantitative measurement of the skin surface roughness would seem most useful, as it would permit a quantification of skin aging, an in vivo analysis of mechanical forces acting on the skin structure, and the detection of abnormalities otherwise not visible. The method described comprises three steps: (1) making a silicone rubber negative replica, (2) making an Araldite positive cast, (3) roughness measurement of the cast with a device commonly used in engineering, which provides quantitative parameters: R_a, R_p, R_t, R_{max} and others. The reliability of each of these steps was checked, and also the absolute need to locate precisely the site of sampling and to know the angle of the scanning direction with the main axis of the limb or the body. The method seems useful for studying aging, either normal or affected by UV rays and other physiopathological events influencing the skin surface.

Key words: Skin surface; Skin replicas; Skin microtopography; Human skin roughness

One of the skin's most salient characteristics is to have a very large surface (in between 1.5 and 2 m^2 for an adult- for a small volume (≃50 cm^3); yet surprisingly, not very much attention has been paid to it. This surface is not flat. It contains incidental features of physiological and possibly pathological significance, which probably express the biomechanical properties of the dermis and overlying tissues. The general relief is difficult to visualize on a child's body without a magnifying glass, but is easier to observe on adults. However, a technique to reveal both qualitatively and quantitatively the microtopography of the skin surface would allow one to study the relationship between skin relief and physiological parameters such as age and sex, the influence of external factors such as sun exposure or UV treatment, or internal ones acting on skin structure. Such a method would be of use to examine the effects of medicines and cosmetic preparations on the skin surface, as well as to detect with precision certain pathological processes such as the evolution of tumours.

To tackle these problems, we have used an apparatus employed in industry to measure the roughness of surfaces. The technique comprises three steps: (*a*) making a silicone rubber negative replica, (*b*) making an Araldite positive cast, (*c*) studying this cast with a roughness-meter which provides quantitative paramenters (r_a, R_p, R_t, R_{max} and others) characterizing the surface.

MATERIALS AND METHOD

Negative replicas were obtained from impressions of the skin, from which casts (positive replicas) were prepared.

The choice of substance with which to make the negative replicas is important. It should be fluid enough to penetrate the furrows and to flow over all the details of the skin surface. It should set at body temperature and in a short time without forming bubbles or artifacts. Finally it should be harmless and easy to apply and remove. For the purpose of this study, three groups of materials were investigated: dental silicone rubber impression materials, a polysulphide dental impression material, and an industrial grade silicone rubber. The results of the comparison are displayed in Table I and it is clear that the silicone rubber impression materials 2, 3, 4 and 5 have advantages over the other materials. The substance found to be most suitable was Silflo®, which was first suggested for use by Sampson (1961) (14). It fulfilled all the above requirements. Silflo is a monomere silicone rubber which transforms into a polymere, when mixed with a catalyst. 1 ml of Silflo was mixed with 2–3 drops of catalyst. The mixture was applied onto the area to be replicated, after having shaved the hairs. A glass microscope slide was placed on the unset material to provide both uniform thickness of the negative replica and firm support so as to prevent the flexible set material from distorting during removal. It is important to indicate the orientation of the replica with regard to the body axis, as it will influence the profile of the relief traced by the roughness meter.

As with the impression materials, cast material should ideally have a low viscosity and be easy to mix. It should also be bubble-free and have a reasonably short curing

Table I. *Properties of impression materials*

Trade name	Supplier	Chemical composition	Characteristics: Flow	Smell	Ease of removal	Current use
1. Kerr–perm-plastic (light)	Kerr. Rog. U.S., Kerr–Europe Germany	Polysulphide rubber	Not good	Not well appreciated	Not easy	Dental impression material
2. Flexicon (injection type)	G.C. Chemical Mfg Co. Ltd., Tokyo, Japan	Silicone rubber	Not good	Acceptable	Easy	Dental impression material
3. Silflo	Flexico Development Ltd.	Silicone rubber	Good	Acceptable	Easy	Dental impression material
4. Verone	Davis Schotlander & Davis Ltd.	Silicone rubber	Good	Acceptable	Easy	Dental impression material
5. Verone G	Davis Schotlander & Davis Ltd.	Silicone rubber	Good	Acceptable	Easy	Dental impression material
6. Silicoset 105 (Batch 21)	Imperial Chemical Industries Ltd.	Industrial Silicone Rubber	Good	Not appreciated	Easy	Industrial use

time at room temperature. The materials investigated were a group of epoxy resins, a two-phase methyl methacrylate replica material (Acrulite), and a calcium sulphate α-hemihydrate ($CaSo_4$). H_2O. It was apparent (Table II) that epoxy resins Araldite MY 753 and Araldite MY 778 with hardener HY 956 proved the most acceptable, as having the advantages of curing at room temperature and the air bubles of the mixture being easy to eliminate.

The cast materials were prepared according to the man-

Table II. *Properties of cast materials*

Trade name	Supplier	Chemical composition	Mixing pro. portion	Characteristics: Ease of mixing	Flow	Air incorporation	Setting temperature
1. Araldite Standard	Ciba-Geigy France	Epoxy Resin	50% Araldite 50% Hardener	Good	Poor	Difficult to eliminate	Room temperature
2. Araldite Nouveau Rapide	Ciba.-Geigy France	Epoxy Resin	50 % Araldite 50 % Hardener	Good	Poor	Difficult to eliminate	Room temperature
3. Araldite CY 219	Ciba-Geigy Duxford	Epoxy Resin	10 parts Araldite 5 parts Hardener 1 part Accelerator	Good	Good	Easy to eliminate	60°C
4. Araldite MY 753	Ciba-Geigy Duxford	Epoxy Resin	80 %Araldite 20 % Hardener	Good	Good	Easy to eliminate	Room temperature
5. Araldite MY778	Ciba-Geigy Duxford	Epoxy Resin	80 % Araldite 20 % Hardener	Good	Good	Easy to eliminate	Room temperature
6. Aliphatic Epon 812	Shell Chemical Co., San Francisco	Epoxy Resin	62 ml Araldite 100 ml Hardener	Good	Good	Easy to eliminate	60°C
7. Acrulite Type GA	Rubert Co. Ltd., Acru Works, Cheadle, Cheshire	Methacrylate Resin	5 parts Polymer 3 parts Monomer	Bad	Not good	No air incorporation	Room temperature
8. Stonehard	The Dental Manufacturing Co. Ltd., London	$(Ca\text{-}So_4)_2$ H_2O	100 parts Stonehard 30 parts water	Good	Poor	No air incorporation	Room temperature

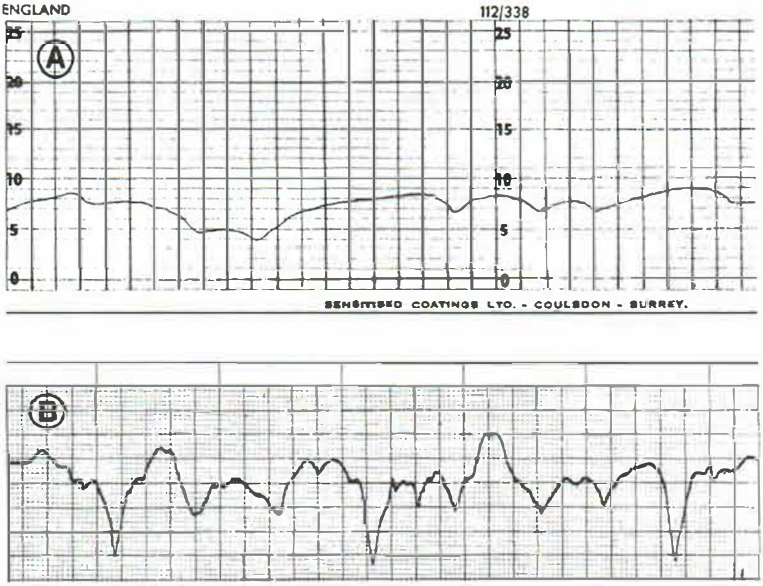


Fig. 1 A. Skin relief profile obtained from Rank Taylor Hobson Talysurf 4, scanned at angle zero. Adult male's abdomen. Magnification: ×65 both vertical and horizontal (photography of an original tracing). *Fig. B.* Skin relief profile obtained from Ferranti Surfcom 38, scanned at angle zero. Adult male's abdomen. Magnification: vertical ×325, horizontal X52 (photography of an original tracing).

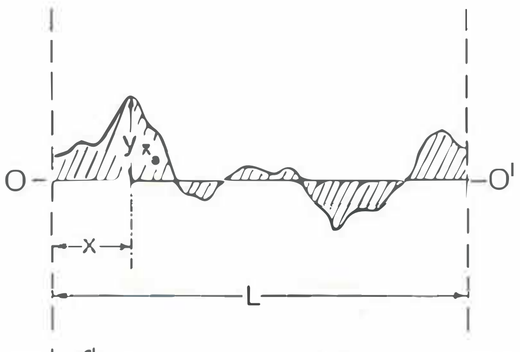
ufacturer's instructions and any air bubbles incorporated during mixing of the epoxy resin were eliminated by placing the mixed material in a container in an ultrasonic cleaner (Dawe Soncleaner type 6442 A or Ponsonic S.C.M.A. PONS) for about 15 min. A wall of freshly mixed Silflo was constructed upon the negative replica so as to enclose an approximately 12 mm diameter area to be reproduced. The prepared epoxy resin was then slowly flowed into the prepared wall, thoroughly coating the impression surface. It is convenient for measurement purposes to have the surface and base of the cast nearly parallel. A jig was constructed which allowed a glass slide to be held parallel to the slide supporting the replica. The replica and cast were stored at room temperature for 24 hours and the hardened cast was separated.

Measurement of surface roughness

There is a wide variety of devices available for measuring the surface roughness of engineering materials and components. In the present investigation we used a Ferranti i Surfcom 38 (Besançon)[1] and a Rank Taylor Hobson Talysurf 4 fitted with a low sensitivity setting (Glasgow).[2] Both devices draw a stylus system across the surface of the cast, which converts vertical displacements into electrical signals. The output is in the form of a chart of the surface configuration (Fig. 1) and the signal may also be processed to provide quantitative measurements of

[1] Ecole nationale Supérieure de Chronométrie et Micromécanique, Besançon.

[2] Division of Metrology, National engineering Laboratory, Glasgow.



$$R_a = \frac{1}{L}\int_0^L |y_x|\, d_x \ ; \quad |y_x| = \text{Height above or below } OO'$$

Fig. 2. Meaning of R_a parameter $R_a = 1/L \int |Y_x| d_x$, where $|Y_x|$ is the amplitude in a given point.
and $\int |Y_x| d_x$ is the hatched area. Accordingly R_a is the hatched area per unit length.

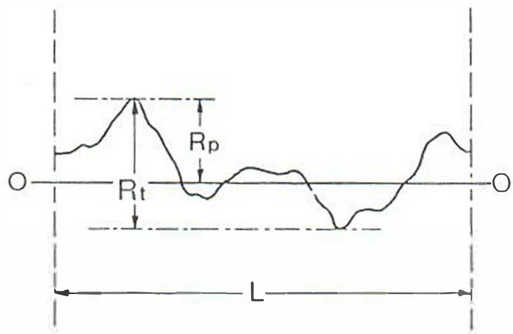


Fig. 3. Meaning of R_p and R_t parameters.

Table III. *Mean and standard deviation of coefficient of variation of R_a and R_p according to body site and direction of scanning*

The numbers of casts examined are given in parentheses

Site	Angle of scan	Coefficient of variation of R_a	of R_p
Abdomen	0°	12.6±6.7 (15)	15.9±4.8 (14)
	90°	12.5±5.4 (5)	17.1±6.2 (14)
Forearm	0°	12.3±6.2 (14)	18.1±9.3 (14)
	90°	17.5±9.4 (14)	20.1±5.2 (14)

the surface roughness (8). The parameters which were used in the present study were the mean Surface roughness (R_a), the maximum peak to valley depth (R_t), and maximum peak height (R_p). The geometrical significance of these parameters is displayed in Figs. 2, 3.

Statistical studies have been performed using Student's t-test for paired comparisons.

RESULTS

I. *Reproductibility of the Method*

1. *Preciseness*

The preciseness of R_a and R_p measurements was assessed by the coefficient of variation (CV) (S.D./mean) % obtained for each of them. The two parameters were calculated on both forearm and abdomen for 14 individuals, with scanning in two perpendicular directions (Table III). The CV values were lower than 0.2 in 78% of cases higher than 0.3 in 6% (Table IV).

The CV of R_a were on the whole lower than these of R_p. This difference was significant with the χ^2-test ($p<0.01$) and was related neither to body site nor angle of scanning.

Table IV. *Distribution of coefficients of variation (CV) of R_a and R_p, irrespective of body site or direction of scanning.*

	CV<0.1	0.1<CV<0.2	0.2<CV
(58) R_a	20 (35 %)	32 (55 %)	6 (10 %)
(56) R_p	7 (12 %)	30 (54 %)	19 (34 %)

2. *Reliabillity*

Replicas are useful for quantitative analysis only if they accurately reproduce the contors of the skin's surface. Two casts (cast I, and cast II) were obtained from the two replicas R_1 and R_2 according to the following sequence

$$\text{skin surface} \rightarrow R_1 \rightarrow \text{Cast I} \rightarrow R_2 \rightarrow \text{Cast II}$$

We performed on cast I and cast II (2 to 17) of parallel scans, at varying angles to the main body or limb axis. The comparison of these 2 casts in the same direction of scanning (Table V) showed that the means of R_a, as of R_p obtained were nearly identical, the difference being not statistically significant ($p>0.3$).

We also compared the casts made from two replicas of the same site on the same person (Table VI). The differences in the mean R_a or R_p of the two casts were not significant ($p>0.5$).

Table V. *Comparison between two casts made from one replica (under the conditions mentioned in text)*

In columns 4 and 6 are indicated the statistical significance (t Student Fisher for paired comparison; in brackets, probability figures)

No. of sample	No. of scans	R_a (μm) Mean ± S.D.	(p)	R_p (μm) Mean ± S.D.	(p)
4-I-0°	10	18.9±2.6	(>0.9)	38.8±6.0	(>0.5)
4-II-0°	17	19.0±1.9		38.1±4.9	
4-I-90°	9	17.8±1.9	(>0.5)	31.1±8.1	(>0.5)
4-II-90°	2	18.3±1.2		27.5±5.3	
5-I-0°	5	17.8±2.7	(>0.3)	39.2±5.1	No difference
5-II-0°	5	16.1±2.9		39.2±5.8	
6-I-0°	8	13.7±2.5	(>0.5)	27.0±6.2	(>0.3)
6-II-0°	8	13.3±2.2		30.0±6.2	

Table VI. *Two casts obtained from two replicas made on the same area (forearm) and both scanned at the same angle, 0°. 4–5: man (38 years old). 60–61: man (20 years old)*

Sample and orientation	No. of scans	R_a (μm) Mean ± S.D.	P	R_p (μm) Mean ± S.D.	P
4-1-0°	10	18.9±2.6	>0.50	38.8±6.0	>0.50
5-1-0°	5	17.8±2.7		39.2±5.1	
60-1-0°	5	16.6±2.0	>0.50	37.2±6.6	>0.50
61-1-0°	6	14.3±1.7		32.6±9.1	

Table VII. *Comparison of R_a values for forearm and abdomen in the same subject*

Case no.	Sex	Age	Angle of scanning	Forearm R_a	Abdomen R_a	p
10C	F	47	0°	14.7±2.4	10.7±1.9	<0.05
			90°	15.45±2.1	10.9±0.9	<0.01
20A	M	11	0°	14.7±1.2	13.2±1.0	<0.05
			90°	13.2±1.4	13.5±1.1	NS
11B	M	11	0°	22.0±3.64	15.5±2.3	<0.01
			90°	17.0±2.9	12.95±1.4	<0.05

II. *Local Factors Influencing the Profile and the Microrelief Parameters*

1. *Topographical variations*

The epidermal grooves which form the characteristic skin surface pattern are irregularly disposed. Therefore different scannings will cross different lines—or, not the same number of lines. This will produce changes in the roughness parameters. To obtain representative values, multiple parallell scans 0.5 mm apart were made allowing mean values and standard deviations to be calculated for the area surveyed. Then two areas (forearm and abdomen) of 3 persons of different ages (Table VII) were compared. The results clearly showed a statistically significant difference in roughness parameters, while any difference was barely visible on photographs produced under the scanning electron microscope.

2. *Directional variations*

The characteristic surface pattern is oriented and accordingly the surface roughness parameters were

Table VIII. *Comparison of R_a and R_t for different angles of scanning*

(+) indicates hte highest value, (−) indicates the lowest value. CV %: coefficient of variation ×100

Body site	Sex	Age	Sample No. Angle	No. of scans	R_a (μm) Mean ± S.D.	R_a CV %	R_t (μm) Mean ± S.D.	R_t CV %
Abdomen	M	28	21-1-0°	5	(−) 12.11±0.4	3.5	(−) 54.4± 5.3	9.7
			21-1-30°	6	12.9 ±1.5	8.1	61.9± 4.5	7.2
			21-1-60°	7	14.3 ±1.6	11.1	66.8± 6.6	9.8
			21-1-90°	5	13.1 ±1.2	9.1	66.2± 6.2	9.3
			21-1-120°	5	(+) 15.1 ±1.5	9.9	(+) 72.6±10.9	14.8
			21-1-150°	5	14.2 ±1.0	7.0	64.0± 6.8	10.6
Forearm	M	11	20-1-0°	6	14.7 ±0.5	3.4	72.9± 3.2	4.3
			20-1-30°	6	13.0 ±0.5	3.8	61.3± 1.0	1.6
			20-1-60°	6	(−) 11.6 ±0.4	3.4	(−) 57.3± 2.0	3.4
			20-1-90°	6	13.2 ±0.6	4.5	64.0± 1.9	2.9
			20-1-120°	6	14.1 ±0.4	2.8	73.4± 2.0	2.7
			20-1-150°	6	(+) 17.1 ±0.9	5.2	(+) 85.7± 3.4	3.8

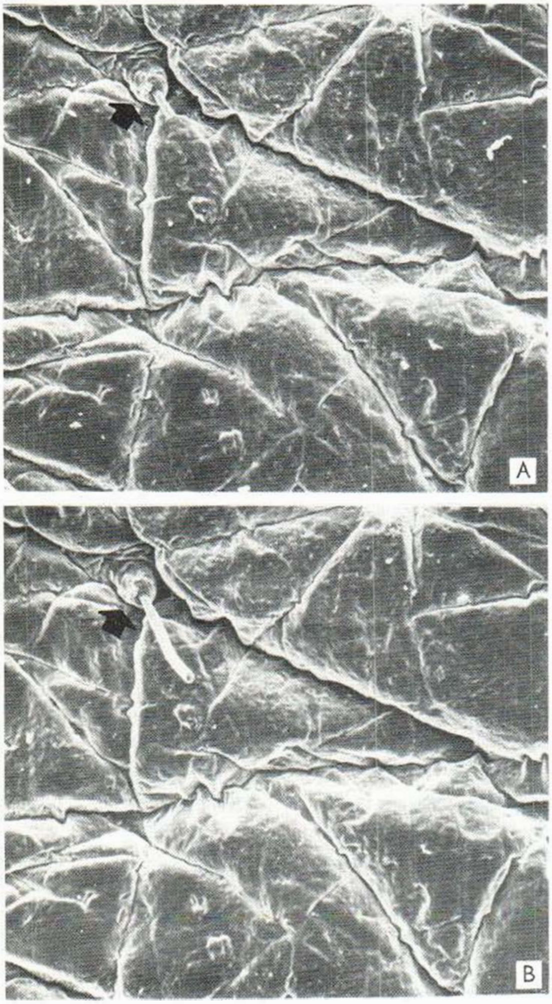


Fig. 4. SEM photographs of two different casts obtained from the same replica. Cast (A) is the original. Arrows point to a hair which was as long in cast (A) as in cast (B) before being accidentally broken.

significantly direction-dependent. The results of multiple parallel scans made at six different angles (0°, 30°, 60°, 120°, 150°) are displayed in Table VIII. The two examples were taken from abdomen and forearm skin on 2 different subjects. For each of them one particular direction gave the highest or the lowest values of R_a, and R_t. The highest values were at 120° to the body axis for the abdomen, and at 150° to the arm axis for the forearm. The minimum values were at 0° for the abdomen and at 30° for the forearm. The differences between the extreme values were significant: $p<0.01$ for the abdomen and $p<0.001$ for the forearm.

DISCUSSION

The microscopic morphology of the surface of the skin has long been studied by dermatologists and anatomists by light microscopy (3, 4, 15) and scanning electron microscopy (1, 2, 5, 6, 7, 9, 10, 11, 16, 18), and described qualitatively as consisting of characteristic skin patterns of epidermal furrows. The distribution of these patterns and their variations with age, sex and disease have also been described (3, 19, 20). But these data, being only qualitative, are of little use in clinical diagnosis or in assessing physiological or therapeutical skin changes. A quantitative approach to this problem gives more valuable information and care serve as a useful tool in experimental dermatology and cosmetology, as it allows the detection and measurement of variations barely visible to the naked eye or even under the scanning electron microscope. The only attempts to produce such quantitative descriptions of the surface roughness of human skin were made by Marks, Nicholls, and Pearse (12, 13) who investigated the internal structure of the stratum corneum obtained by stripping, using cyanoacrylate. In this case, what was looked at was not the skin surface itself, but the interface stratum corneum layers after stripping some of them. The most convenient and satisfactory method of examining the skin surface structure is by the production of replicas which accurately reproduce the structure of the stratum corneum.

Among many impression and cast materials commercially available, the combination of Silflo silicone rubber impression material and Araldite MY 753 or 778 was shown to be the most reliable, qualitatively and quantitatively, for reproducing the skin's surface (Fig. 4). The replica technique has the advantage of being rapid and harmless even for very young people. It can be repeated many times on the same area. The casts can be conserved for long periods of time wothout any risk of modification of the surface pattern. The precision was satisfactory, as shown by the good values of the coefficient of variation (Table III). The reliability was good (Tables V and VI) when the protocol of the method was respected, especially the parallelism of the superior and inferior surfaces of the cast and the absence of air bubbles in the Silflo or the Araldite.

The differences observed between casts made from the same replica (Table V) or from different replicas of the same cutaneous area on the same individual (Table VI) proved non-significant. In contrast, we found highly significant differences between forearm and abdomen skin relief in the same person (Table VII). In a given region of the body the figures were individual-dependent.

The surface parameter values changed according to the angle of scanning (Table VIII). Consequently it is necessary to scan at different angles and find which direction should be adopted in order to obtain the most specific results. How many scans should be made in the chosen direction? Is it suitable to get a coefficient of variation lower than 0.2. This result could sometimes be obtained by only two scans, but performing 5 scans systematically would afford good precision when the coefficient of correlation could not be calculated immediately. Which of the parameters is the more important and gives better reliability? R_a is the more complete one as it reflects the mean amplitude of peaks and troughs. R_t probably represents the adnexal pores, which are not numerous, and accordingly gives serious standard errors. Nevertheless these two parameters seem complementary. But a correct interpretation would require a better knowledge of their anatomic nature, which is the objective of a work presently in progress in our Laboratory.

AKNOWLEDGEMENTS

We are grateful to Mr A. Harvey and J. Beattie of National Engineering Laboratory, Glasgow, and Mr R. Stepourgine and F. Bigle of the Ecole Nationale Supérieure de Chronométrie et de Micromécanique, Besançon, for providing us with every facility and allowing us to use their apparatus and for their kind advice.

The contribution of S. Makki was supported by a grant from the Institut National de la Santé et de la Recherche Médicale.

REFERENCES

1. Barnes, I. E.: Techniques for the replication of skin surface. A new method. Br J Dermatol *89:* 277–283, 1973.
2. Bernstein, E. O.: Skin replication procedure for scanning electron microscopy. Science *166:* 252–253, 1969.
3. Chinn, H. D. & Dobson, R. L.: The topographic anatomy of human skin. Arch Dermatol *89:* 267–273 1964.
4. Collins, K. J., Sargent F. & Weiner J.: Excitation and depression of eccrine sweat glands by acetylcholine, acetyl-β-methylcholine and adrenalin. J Physiol *148:* 592–614, 1959.
5. Fujita, T.: Scanning electron microscopy of the skin using celluloïd impressions. Arch Histol Jpn *30:* 321–326, 1969.
6. Garber, Ch. A. & Nightingale C. T: Characterizing cosmetic effects and skin morphology by scanning electron microscopy. J Soc Cosmet Chem *27:* 509–531, 1976.
7. Goldmann, L., Vahl, J. & Rockwell, R. J., Jr: Replica microscopy and scanning microscopy of laser impacts of the skin. J Invest Dermatol *52:* 18–24, 1969.
8. Harvie, A. & Beattie, J. S.: Surface texture measurement. The Production Engineer (G. B.) 25–29, Feb. 1978.
9. Hashimoto, K. & Kanazaki, T.: Surface ultrastructure of human skin. Acta Dermatovener (Stockholm) *55:* 413–430, 1975.
10. Johnson, C., Daider, R. & Shuster, S.: Surfaces appearances of the eccrine sweat duct by scanning electron microscopy. Br J Dermatol *83:* 655–660, 1970.
11. Kuokkanen, K.: Replica reflexion of normal skin with disturbed keratinization. Acta Dermatovener (Stockholm) *52:* 205–210, 1972.
12. Marks, R. & Pearse, A. D.: Surfometry: A method evaluating the internal structure of the stratum corneum. Br J Dermatol *92:* 651–657, 1975.
13. Nicholls, S. & Marks, R.: Novel techniques for the estimation of intracorneal cohesion in vivo. Br J Dermatol *96:* 595–602, 1977.
14. Sampson, J.: A method of replicating dry or moist surfaces for examination by light microscopy. Nature *191:* 932–933, 1961.
15. Sarkany, I.: Method for stydying the microtopography of the skin. Br J Dermatol *74:* 254–259, 1962.
16. Sarkany, I. & Caron, G. A.: Microtopography of the human skin. Studies with metal shadowed replicas from plastic impressions. J Anat *99:* 359–364, 1965.
17. Sarkany, I. & Gaylarde, P.: A method for demonstration of sweat gland activity. Br J Dermatol *80:* 601–605, 1968.
18. Sarkany, I. & Gaylarde P.: A method for demonstration of the distribution of sebum on the skin surface. Br J Dermatol *80:* 744–746, 1968.
19. Verbov, J.: Hypohidrotic (or anhidrotic) ectodernal dysplasia. An appraisal of diagnostic methods. Br J Dermatol *83:* 341–348, 1970.
20. Verbov, J.: Palmar ridge appearances in normal newborn infants and ridge appearance in relation to eccrine sweating. Br J Dermatol *93:* 645–648, 1975.

Based on a paper read at the Société Française de Dermatologie et Syphiligraphie, Paris, May 1978.

Received November 27, 1978

S. Makki, M.D.
Clinique Dermatologique Universitaire
Faculté de Médicine et Pharmacie
F-25030 Besançon Cedex
France