New Evaluation system of Cosmetic Effects on Morphology of Skin Surface Using TSRLM with Image Analyser

Jong il Kim, Joa Hoon Lee, Yoo Young Lee, Chang Kew Kim
Pacific Chemical Co. Ltd., R & D Center

SUMMARY

Image analyser was used to understand the condition of skin surface and to evaluate the efficacy of cosmetic treatment. It was unsatisfactory to analyse skin surface structure although several methods using image analyser had been presented.

We developed the new system composed of image analyser and Tandem Scanning Reflected Light Microscope (TSRLM) having the remarkable optical sectioning property as image input device. By using this new system, we quantitatively measured the change of skin surface, the depth and width of furrow in micron unit, resulted by cosmetic treatments. And also three dimensional image of skin was reconstructed with serial sectioned images, which were captured through TSRLM, for better understanding of the effect of cosmetic treatment.

It was found that skin relief was more easily understood and the change of skin surface caused by cosmetic treatment was more accurately measured by using this system. In addition, we was also aware of the possibility of in vivo direct measurement of skin furrow without replica.

It was conceivable that our system could be applicable for the study of cosmetic effects further.

I. INTRODUCTION

The quantitative analysis of skin cutaneous relief has been performed by using noninvasive instrumental methods such as scanning densitometry, stylus profilometry,

scanning electron microscopy and image analysis. Among these, image analyser has recently been used with macroviewer, stereomicroscope and conventional light microscope as image input device for image analysis(1－3). However, above input devices have several problems, for example, macroviewer : limited magnification power, stereomicroscope and conventional light microscope : out-of-focus structure. These effects can be greatly reduced by a scanning confocal light microscope(4).

Tandem scanning reflected light microscope(TSRLM), one type of scanning confocal light microscope, having the remarkable optical sectioning property, has been used as input device to evaluate the surface topography of living tissues, metal and polymers with image analyser.

With introducing TSRLM into cosmetic field for the first time, it was possible to measure the real change of skin surface, the depth of micron unit, and to make three dimensonal reconstruction with serial sectioned images for the better understanding of cosmetic effects quantitatively.

Furthermore, we have attempted to analyse the skin surface directly by using this system without artifact of replication (repeated replicas on one site may alter or remover some surface characterisitics).

Ⅱ. MATERIALS AND ANAYTICAL METHODS

Subjects

32 korean women without apparent skin disease in the twenties age (average is 24 years old) were served as volunteers in this study.

Testing Condition

Relative humidity and temperature were monitored in the laboratory area (56±5 % RH, 19±2℃).

Replication Procedure

Silicone rubber impressions (Silflo, Flexico Developments Ltd.) was used as the negative replication materials of the skin surface. Replicas were taken on the crow's foot (around the eye) and the forearm(2, 5).

Outline of the Method for Measrement and Analysis

Fig. 1 shows experimental scheme to analyse the morphology of the skin surface and its replica.

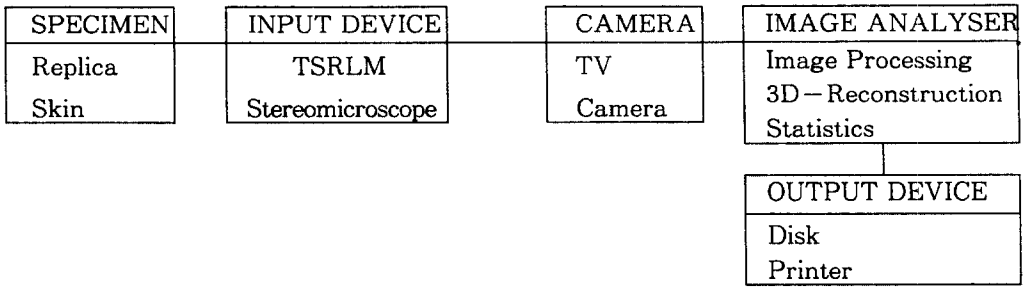


Figure 1. The outline of image processing.

The surface images of skin replicas were captured by input devices, TSRLM and STEREOMICROSCOPE, and photographed with TV camera. The analysis of input images was done with the image analyser.

STEREMICROSCOPE : WILD
TERLM : Confocal 2002, STROJIMPORT
Objective 10X(NA 0.5), Ocular 10X
TV CAMERA : BOSCH TYK 91A TV
IMAGE ANALYSER : JOYCE LOEBL Ltd. MAGISCAN

Principles of TSRLM and Transportation of Optical Serial Sectioned Images

The TSRLM is a microscope with remarkable optical sectioning properties, which provides both high contrast images of surfaces and pictures of the internal structure of translucent specimens. The basic principles of TSRLM are that the incoming light is provided as series of mini-beams which scan the field of view and illuminate corresponding patches in the plane of focus more intensely than out of focus layers.

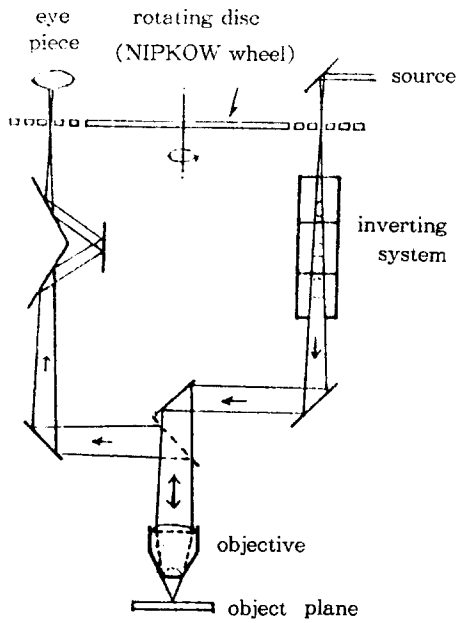


Figure 2. Diagram of the optical layout of TSRLM(8).

Reflected ligth from these patches is imaged on to Nipkow's aperture which are scanning in the focal plane of the eyepiece. The array of apertures which chop the illuminating beam is identical with the array on the observation side (6, 7). The scanning is done by Nipkow Disc, which is the only scanning device currently rapid enough to permit real time imaging (Fig. 2).

The scanning, illuminating apertures are imaged by the objective lens in the focus on plane. Reflected light from the patches in this plane is imaged back into corresponding apertures which also lie in the intermediate image plane of the objective. All other reflected light from out of focues layers and light scatterred from optical surfaces in the microscope is intercepted by the solid potions of the aperture array or by light traps in the microscope head.

The optical sectioned images of skin replica were serially captured, focussed up every 3 microns. The captured serial images were processed and reconstructed with 3 −dimension (Fig. 3).

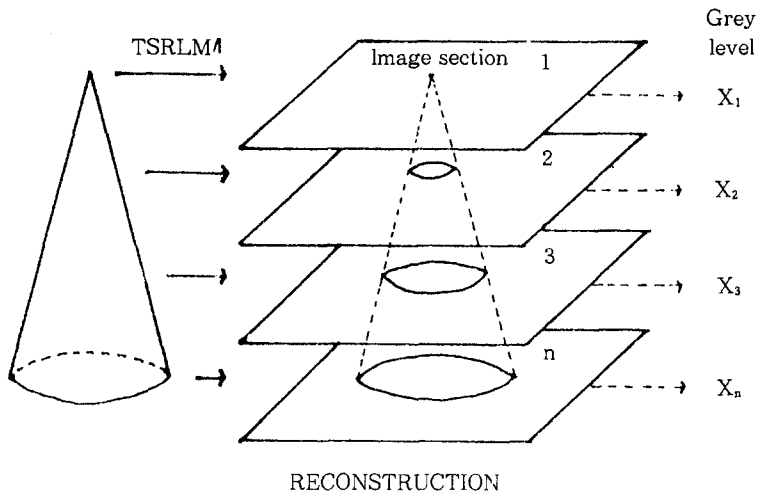


Figure 3. The diagram of 3D reconstruction of optical section images produced by TSRLM.

The depth of skin furrow was measured by micrometer with turning fine adjustment. And also the volume of furrow in 3D-reconstructed image could be calculated into the integrated optical density (IOD) value as equation 1.

$$\text{Volume} = \sum_{\text{area}} \text{Height} \propto \sum_{\text{pixel}} \text{Grey Level}$$

$$\text{Optical Density} = \text{Log}\left(\frac{1}{\text{Grey Level}}\right)$$

$$\text{Integrated Optical Density (I O D)} = \sum_{\text{pixel}} \text{Log}\left(\frac{1}{\text{Grey Level}}\right) \propto \frac{1}{\text{Volume}} \quad ----①$$

Figure 4. shows optical sectioned images of skin replica in each focal plane and 3D-reconstructed image of them.

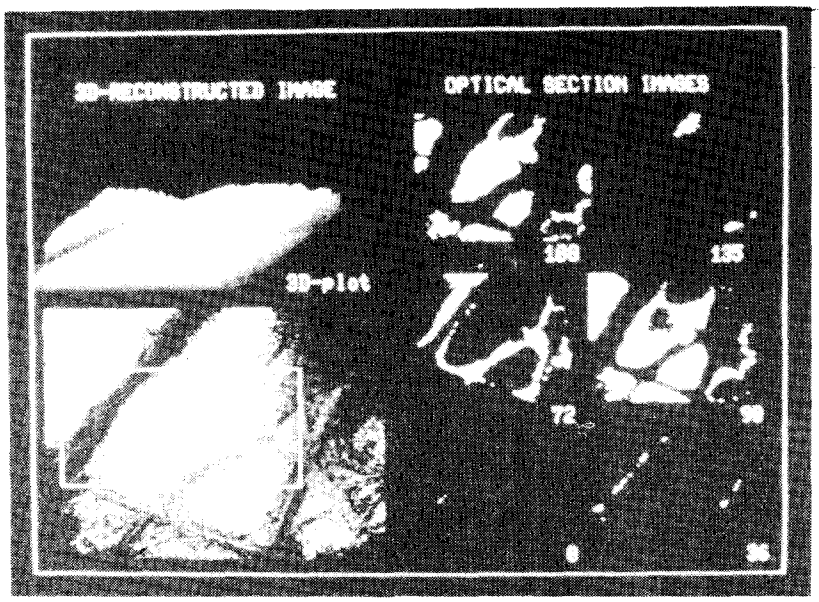


Figure 4. 3D-Reconstructed image (left) and optical sectioned images of skin replica (right) through TSRLM. The number in optical sectioned image means height of skin replica furrow in microns.

Another roughness parameter, width parameter of furrows was calculated by measuring detected area(Da) which means the total number of pixels having grey value more than 1(9).

The Processing of Image Captured by Stereomicroscope

The images of skin replicas on the stereomicroscope stage were captured by illumination of light with 24 angle. With the same method, image data from 8 directions, rotated at 45 angle, were fed into image analyser for each replica(10). Input image was processed and measured by image analyser with grey level (Fig. 5). Typical parameters of skin roughness are as follows (Table 1).

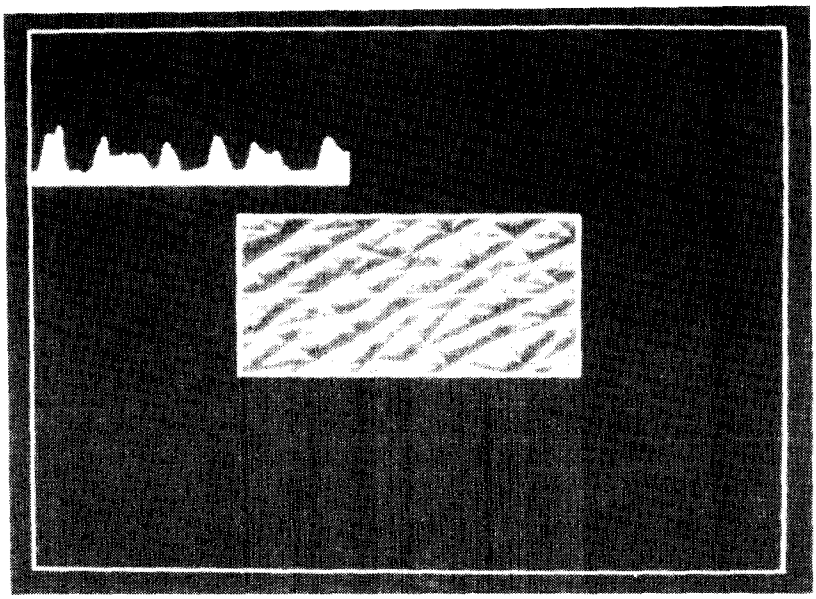
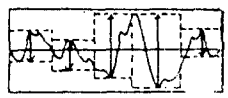


Figure 5. Image of skin replica through the stereomicroscope.
The left profile is grey profile of straight line
on the image.

Table 1. The Definition of Used Roughness Parameters (5).

PARAMETERS	MEANINGS	GRAPHIC DEFINITIONS
Ra	Mean Surface Roughness $Ra = \frac{1}{\ell}\int_0^{\ell} \lvert Ly(x) \rvert \, dx$	
Rz	Mean Depth Roughness $Rz = \frac{Z_1 + Z_2 + \cdots + Z_n}{n}$	
Ry	Ry = Peak(Max.) − Valley(Min.)	
Sa	Shadow Area $Sa = \frac{\text{Detected Shadow Area}}{\text{Total Area}}$	
Sm	Average Distance between Peaks $Sm = \frac{S_1 + S_2 + \cdots + S_n}{n}$	

Ouality of the Image

To examine the quality of the image data, the new evaluation system using the TSRLM and the Stereomicroscope method were compared(fig. 6)

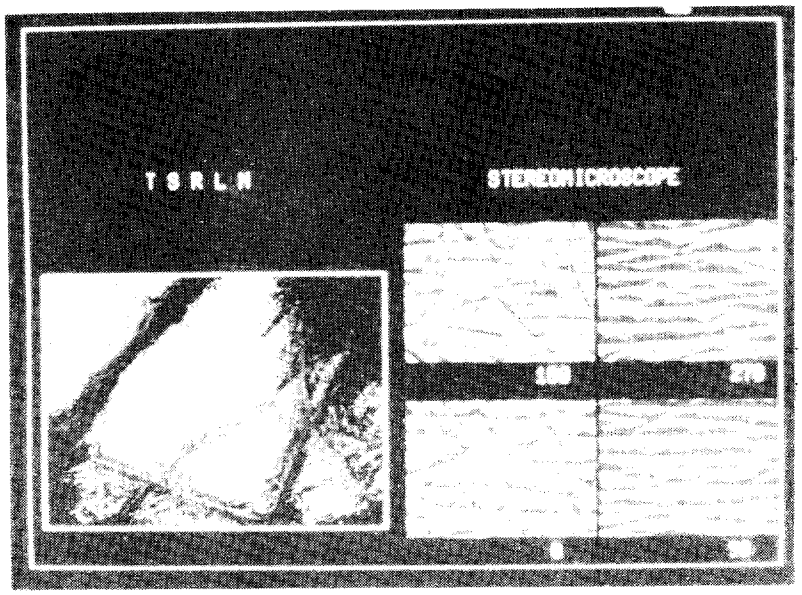


Figure6. Comparision of image data through TSRLM (left) and stereomicroscope (right). In left image the grey level increase according to the height of skin replica furrow. In right image the number means the angle of rotation on the stage of stereomicroscope.

Stereomicroscopic system has some disadvantages in accuracy beacuse roughness parameter values can be changed by incident light angle, rotation angle and reflection. However, the new evaluation system using the TSRLM can measure not only the real depth of furrow, but also the volume of furrow from calculating of integrated optical density through 3D-reconstruction of image data.

Measurement of Skin Hydration by Corneometer

The hydration value of the experimental sites was measured by Corneometer CM 420 (Schwarzhaupt)

Ⅲ. EXPERIMENTAL AND RESULTS

Measurement of the change of skin furrow

To investigate the effect of the cream treatment on the skin furrow change, replicas of crow's foot were taken from 20 korean women before and after 2 hr of the cream treatment. The skin roughness of replicas was measured by TSRLM and Stereomicroscope with Image Analyser.

TSRLM

Typical examples of 3D-reconstucted image were represented in Fig. 7. The results of analysis of skin furrow before and after the cream treatment are shown in Fig. 8. Fig. 7 and 8 appear that the treatment of cream decrease the depth and volume of the skin furrow significantly.

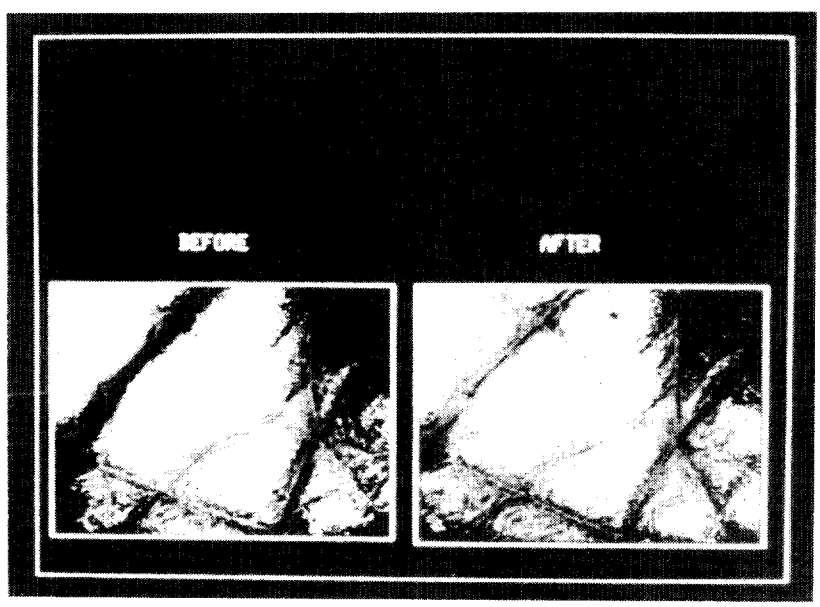


Figure 7. Typical examples of 3D-reconstructed image of skin replica before and after treatment.

STEREOMICROSCOPE

The results of analysis of skin furrow before and after the cream treatment are shown in Table 2.

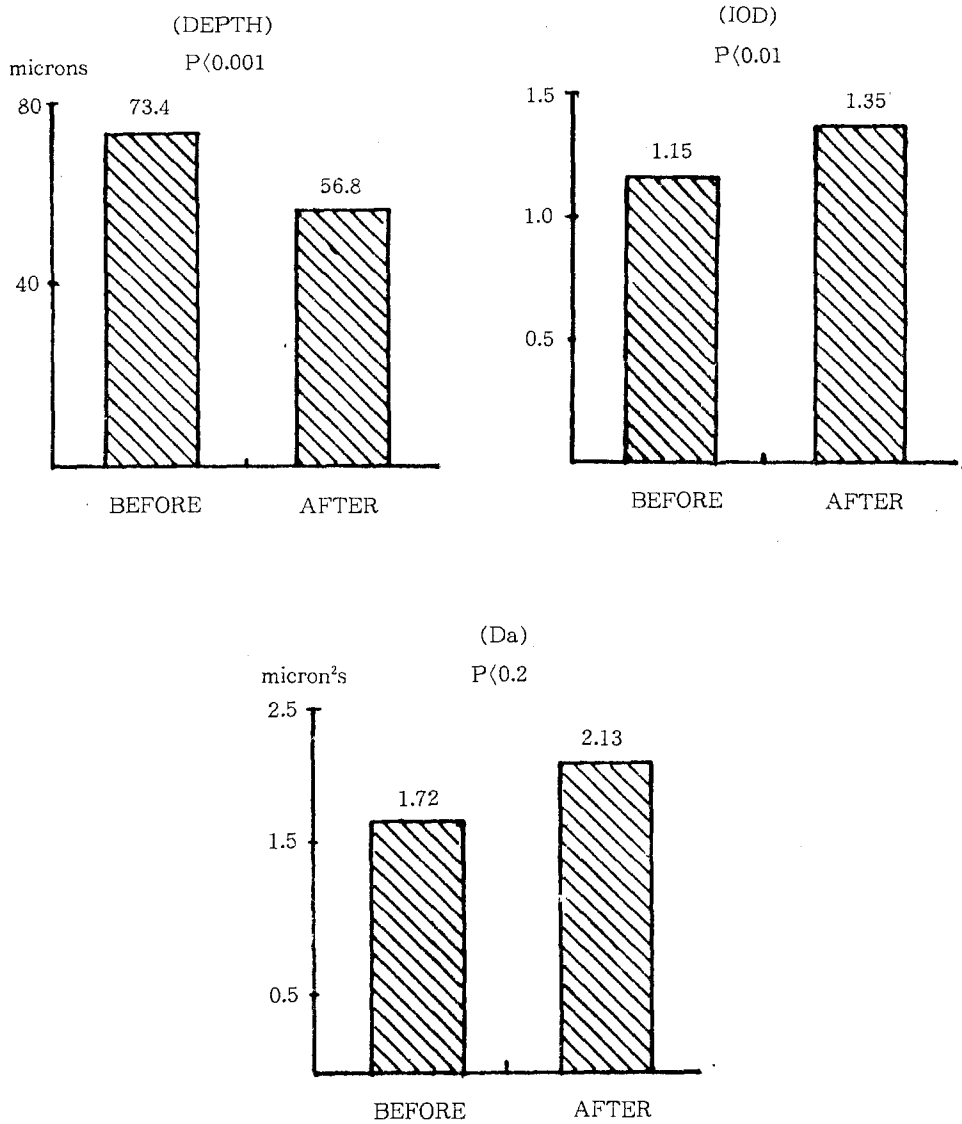


Figure 6. The depth, volume and width skin furrow before and after cream treatment.

a) Depth of furrows in micron unit.

b) Volume of furrows in integrated optical density.

c) Width of furrows in square of microns.

Table 2. The results of skin roughness parameters before and after cream treatment through stereomicroscope.

Parameters	Roughness value (Mean ± SD)		P-value
	Before	After	
Ra	3.27±0.90	2.96±0.70	0.017
Rz	2.66±0.54	2.34±0.44	0.001
Sa	2.49±0.72	2.38±0.63	0.450
Ry	21.29±4.19	20.15±3.54	0.087
Sm	9.34±0.60	8.95±0.54	0.003

From the result of analysis of roughness parameters with the same replica, it can be seen that Rz, Sm, Ra parameters are more significant in respect to accuracy than Sa, Ry.

The Depth of Skin Furrow and Skin Hydration

It was conducted on the forearm of 13 normal volunteers to study the effect of moisturizer solution (Table 3) on the skin roughness. Measurement of hydration value and replication procedure were performed before and after 2 hr of moisturizer solution treatment on the same sites.

Table 3. Formula of Moisturizer Solution

Substance	Content(%)
Propylene Glycol	7.0
Glycerine	14.0
Hyaluronic Acid-Na (0.5% sol'n.)	7.0
D. I. Water	to 100

HYDRATION VALUE OF SKIN

The results of measurement of skin hydration value change by the moisturizer solution treatment are shown in Fig. 9.

DEPTH OF SKIN FURROW

The analytical results of the depth of skin furrow by moisturizer solution treatment are shown in Fig. 10.

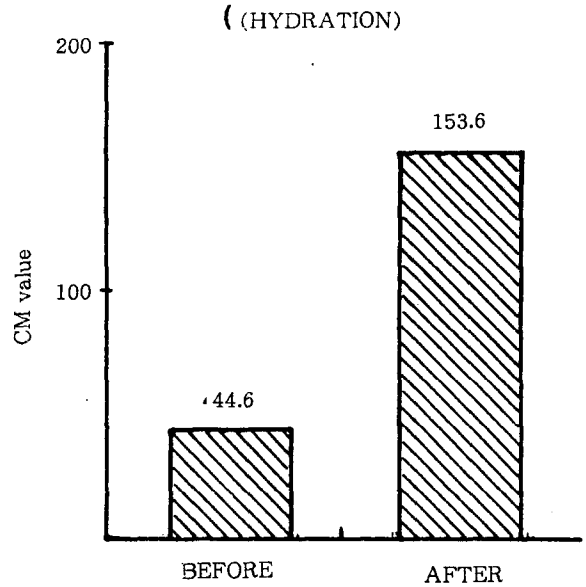


Figure 9. The skin hydration value before and after treatment.

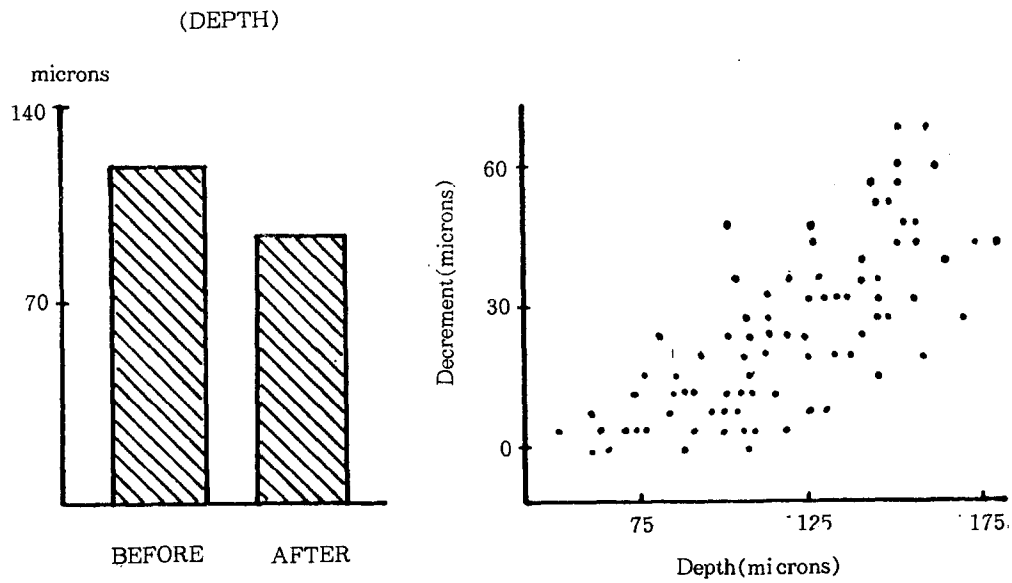


Figure 10. a) Depth of furrows before and after the treatment.
b) Dot plot of depth of furrow before and decrement amount after the treatment.

The change of depth of skin furrow by the treatment increased in proportion to the depth of furrow before treatment($r=0.727$).

RELATIONSHIP BETWEEN CHANGE OF DEPTH OF SKIN FURROW AND THAT OF HYDRATION VALUE.

The change of depth of skin furrow increased in accordance with that of hydration value($r=0.778$) (Fig. 11). It seems that depth of furrow is influenced proportionally by the hydration of skin.

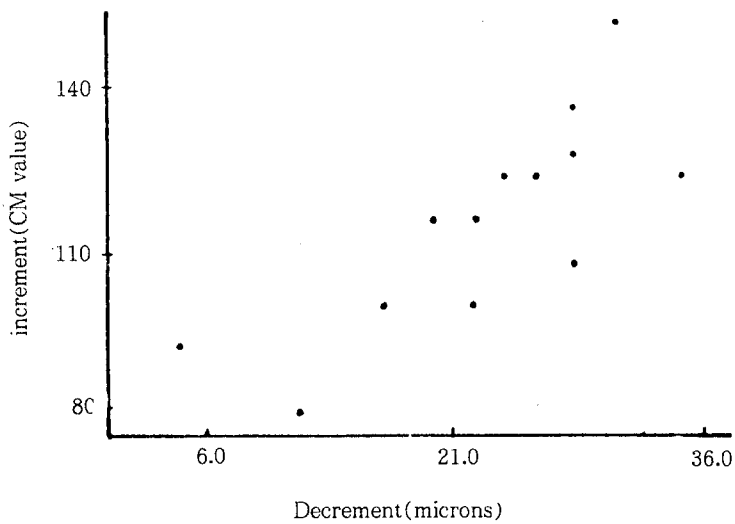


Figure 11. Increment of a hydration and decrement of the skin furrow depth by moisturizer solution.

Direct Measurement of Depth of Skin Furrow without Replica

To Minimize any movement of the furrow skin surface to be measured, the test sites were marked and attached to specially designed holder on the stage of TSRLM with double stick disc.

VALIDATION OF DIRECT MEASUREMENT

This study was designed to confirm its consistency of in vivo measurement. The depth of four furrows was measured 5 times. It can be proved that this method give a good reproducibility of standard deviation within 6% of mean depth (Table 4).

Table 4. The depth of furrow measured by direct method.

Furrows	Depth(MEAN ± SD, microns)
1	215.2±9.7
2	208.6±11.1
3	211.0±9.1
4	155.4±8.7

(n = 5)

3D-RECONSTRUCTION OF DATA IN VIVO MEASUREMENT

Fig. 12 shows that 3D-reconstruction of image data in vivo measurement through 10 microns unit of optical sectioned image.

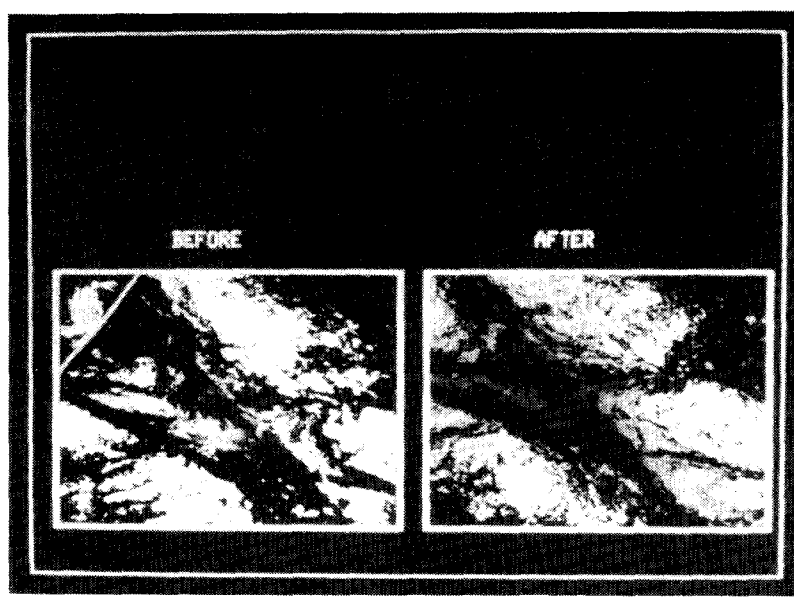


Figure 12. 3D-Reconstruction image of skin furrow before and after cream treatment

THE EFFECT OF CREAM TREATMENT ON DEPTH FURROW IN VIVO

The change of depth of furrow was measured before and after 2 hr cream treatment. Table 5 shows the effect of cream treatment on depth of furrow has statistically significant meaning.

Table 5. The depth of skin furrow before and after cream treatment without replica(* : P〈0.05).

	Depth (MEAN ± SD, microns)
Before	203.3 ± 5.7
After	180.2 ± 12.6*

Ⅳ. DISCUSSION

This study was designed to develop the new evaluation system for measuring the real depth of skin furrow. Many efforts have been paid for comprehending the skin surface configuration by using stylus profilometry, scanning densitometry, SEM and image analyser. However, these methods have the several problems : replication, out-of-focus image, time-consuming, and so forth. Quantitative evaluation of microscopic image has been complicated by the effect of out-of-focus structure on the final image. These effects can be greatly reduced if the conventional light microscope is replaced by a scanning confocal light microscope(4). TSRLM, one type of scanning confocal microscopes, provides an almost real time image which is essential if the biological specimen is changing rapidly and can perform 3D-reconstruction for the optical sectioned images (11−13).

By using the new system, TSRLM and Image analyser, the skin configuration was measured and composed the 3D-reconstruction in aspect of skin surface changing pattern.

Roughness parameters of stereomicroscope method are shown in more unexpected percentage than those of TSRLM. The results obtained comparing TSRLM and Stereomicroscope as the input device suggested that roughness parameters of TSRLM were more accurate than these of stereomicroscope(Table 6).

Table 6. The percentage of unexpected data.

Method	Parameter	No. of Sample with unexpected data*/Total No. Percentage
New Old	Depth	0.0
	Volume	7.7
	Rz	11.8
	Ra	29.4
	Sa	35.3
	Ry	41.2
	Sm	11.8

* : The unexpected data is that the roughness parameter value of after treatment was higher than before.

The decrement of depth and volume of furrow by cream treatment was 16.5 microns($P<0.001$) and 0.20 ($P<0.01$) as IOD, respectively. In addition, skin furrow width parameter, Da, increased with 0.410, but there are no signiticant difference($P<0.2$). It seems that this results are in accordande whth the reports of S. Makki (2) and S. Nicholls(14). It is conceivable that the depth of furrow are more influenced than other parameters, volume and width parameter by cream treatment. Therefore we introduced the depth of furrow as the best roughness parameter(Fig. 7).

In order to investigate the effect of skin hydration on the depth of furrow, the moisturizer solution was treated to the forearm. As shown in Fig. 9－10, the change of depth of skin furrow by treatment increased proportionally to the depth of furrow before treatment and also the depth of furrow was more influenced by the amount of skin hydration. In this system, major problem is movement of skin specimen to be measured, caused by internal and external factors(ex. blood flow, breathing, tension, and so forth). However, the reproducibility of four measurements is excellent as shown in Table 4. From confirming the reproducibility in direct method, the change of depth of skin furrow could be measured.

Furthermore, we suggest that it is particularly helpful when assessing skin surface active materials to use a combination of replicas and in vivo direct mesurement. In this way, it is possible to compare what is taking place at the surface with changes of some cell layers.

We hope to employ this system described to evaluate the effects of sunlight, age, other physiological parameters and cosmetic treatments thought to have a hydration action on the skin and to correlate these effects with changes in the pattern of eidermal growth and maturation.

V. REFERENCE

1. Glove, G. L. and Glove, M. J., Objective methods for assessing skin surface topogrphy noninvasively in "Cutaneous Investigation in Health and Disease". Marcel Dekker(1989).

2. Makki, S. and Agache, P., Statistical analysis and three dimensional representation of human skin surface. J. Soc. Cosmet. Chem., 35, 311－325(1984).

3. Hayashi, S., Mimura, K. and Nishijima, Y., Changes in surface configuration at the

skin caused by aging and application of cosmetics : Three dimensional analysis according to a new system based on image analysis and Fourier transformation. Int. J. Cosmet. Sci., 11, 67－85(1989).

4. Schatten, G. and Pawley, J. B., Advances in optical, confocal and electron microscopic imaging for biomedical researchers. Science, 239, Part Ⅱ, G164(1988).

5. Cook, T. J. and Craft, T. J., Topographics of dry skin, non-dry skin, and cosmetically treated dry skin as quantified by skin profilometry. J. Soc. Cosmet.Chem., 36, 143－152 (1985).

6. Boyde, A. Confocal optical microscopy, Microscopy and Analysis

7. Petran, M., Hadravsky, M. and Boyde, A., The tandem scanning reflected light microscope. Scanning, 7, 97－108(1985).

8. Xiao, G. O. and Kino, G. S., A real time confocal scanning optical microscope. Proc. 4th Int. Symposium on Optical and Optoelectronic App. Sci. and Eng., (1987).

9. Joyce Loebl, Image Analysis : Principle and Practice. Short Run Press, Great Britain (1985).

10. Curcuff, P., de Rigal, J. and Leveque, J. L., Skin relief and aging. J. Soc. Cosmet. Chem., 34, 177－190(1983).

11. Carlssen, K. and Aslund, N., Confocal image for 3D digital microscopy. Applied Optics, 26, 3232－3238(1987).

12. Hamilton. O. K. and Wison, T., Three dimensional surface measurement using the confocal scanning microscope. Applied Phys., 627, 211－213(1982).

13. Brankenhoff, G. J., van der Voort, H.T.M., van Sprosen. E.A., Linnemans, W. A.M. and Nanninga, N., Three dimensional chromatin distribution in neuroblastoma nuclei shown by confocal scanning laser microscopy. Nature, 317, 748－749(1985).

14. Nocholls S., King, C. S. and Marks, R., Short term effects of emollients and a bath oil on the stratum corneum. J. Soc. Cosmet. Chem., 29, 617－624(1978).