

COSMETIC EFFICIENCY ASSESSMENT THROUGH CONFOCAL LASER SCANNING MICROSCOPY

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Summary

Nowadays, the determination of human skin microtopography is currently carried out by profilometric and surfometric methods which are based on mechanical or optical conceptions. The skin microstructure assessment is performed through a skin replicas made of silicone rubber (Silflo®) or with cast in epoxy resin (Araldite®). Profilometric and surfometric methods allow to quantify skin relief by using parameters such as Rtm, Sm ... Scanning electron microscopy shows images and gives qualitative information.

In the other hand the confocal scanning microscope (CLSM), gives simultaneously images of the skin surface associated with quantitative measurements of the microtopography. With this apparatus, it is easy to perform a skin images with an assessment of the microstructure before and after applications of topical medicine or cosmetic for evaluating their efficiency. It is possible to work in vitro by using replicas, or in vivo, directly on the skin surface. The major inconvenient of this technique is a limited area to explore (3-5 plateaus). We are progressing in solving this problem by using a special device for increasing the studied surface. Moreover, it is indispensable to study the same area (same plateaus and same furrows). For this reason it is necessary to localize exactly the same area (1.2 x 2mm) before and after a product final application, whatever the period extend of treatment.

Riassunto

La determinazione della microtopografia della cute umana viene normalmente effettuata mediante l'utilizzazione di metodologie meccaniche (profilometria) o ottiche (surfometria).

La microstruttura della pelle viene replicata con l'utilizzazione di resine siliciche (Siflo®) o epossidiche (Araldite®). Per ottenere valori quantitativi è necessario utilizzare assieme a queste tecniche, la microscopia elettronica a scansione.

Con la microscopia a scansione confocale (CLSM) è possibile invece ottenere contemporaneamente sia le immagini che i valori quantitativi sia "in vitro" che "in vivo". Il vero inconveniente di questa

metodica è che si possono controllare soltanto piccole aree. Il nostro gruppo di lavoro sta cercando di risolvere questo problema.

E' però indispensabile che le aree controllate prima e dopo il trattamento siano sempre rigidamente le stesse, qualunque sia la durata del trattamento utilizzato.

INTRODUCTION

Cosmetics are daily used by a large number of people of different ages. For this reason, it seems necessary to control the efficiency of these products and to verify their attributed good characters as skin smoothness, skin hydration, etc... In addition, it is important to protect the consumers from false and misleading or exaggerated claimed qualities related to some cosmetic products. A cosmetic efficacy is credible by the determination of the skin restoration after a cosmetic topical application during the indicated period of time.

The aim of this work was to establish a simple technique for a precise location of the skin surface to study, followed by visualization and assessment of the skin microtopographical relief after a cosmetic application. Using a replica technique associated with a confocal laser scanning microscopy, a simultaneous imaging and a quantitative determination of the human skin microtopography were performed.

MATERIALS AND METHODS

1. Replication of the skin surface

An impression of the skin microstructure was taken by using a silicone rubber material (Silflo®, Flexico Development Ltd, London, GB). The area to be replicated was shaved and exactly localised. Two ml of the fluid impression material, mixed with 3-5 drops of a specific catalyst, was applied on the skin surface of the chosen area. A glass microscope slide was placed onto the unset material in order to provide uniform thickness, and to facilitate the removal of the skin surface replica (Makki et al, 1979).

2. The confocal laser scanning microscope (CLSM) (Figure 1)

The CLSM employed in this work was the TCS

4D of LEICA (Paris-Lyon). It has a resolution power of $0,2 \mu\text{m}$ in the plane X/Y and a $0,4 \mu\text{m}$ in the Z axis for a wavelength of 488 nm. This apparatus offers a good bi-dimensional image in the two axis X and Y and in the three axis X, Y and Z, giving a three dimensional representation.

The specimens are illuminated through imaging lenses. A diffraction limited light spot is produced within the sample with a beam source (a). A pinhole (b) focuses the spot. Re-emitted light is deflected by a beam splitter (c) and is detected by a photomultiplier (d). In order to produce an image, the light point is moved in X and Y directions by a mirror driven by two galvanometers over a defined area of the sample. A high precision focusing stage and a computer allow the user to produce whole series of sectional images and store informations on optical disks. Topographic measurements, as the distance between two points or the width of plateaus surfaces and the depth of furrows, can be easily calculated. (Corcuff and L  v  que, 1993 ; Rajadhyaksha et al, 1995 ; Sheppard and Shotton, 1995 ; Paddock, 1999).

EXPERIMENTAL

1. Location of the skin surface (Figures 2a, 2b)

A simple accurate marking method, using a special marker, was performed for indicating easily the skin surface area to replicate and to reproduce exactly, after 60 days and even more.

2. Procedure

In this work, the skin surface studied was an area near the right eye of a volunteer female, 45 year-old. The skin surface was replicated with Silflo® before applying a moisturizer (c). Replicas were taken from the same skin surface. After one month of the cosmetic product (c) daily

application on the replicated surfaces, according to the instructions written on the cosmetic bottle, the replicas were analyzed qualitatively and quantitatively through the CLSM. The apparatus moves the sample from the top reference to the bottom reference which have been previously manually fixed and memorized each point of the surface. The depths of furrows were calculated by a computer program, sectioning optically the image from the top to the bottom, in ten layers. The distance between two points or two lines (furrows) could be measured by the same program. The acquisition was automatically performed. A clear image could be given by the CLSM of the skin surface representing 4-5 plateaus, separated by crossing furrows.

RESULTS

The surface of the area (plateaus and furrows) treated by the cosmetic was relocated easily after 30 days. Photographs of the skin surface replicas were taken, with the CLSM, before and after the moisturizer application. The restoration of the skin surface was clearly noticeable from the images. There was a diminution of furrows (A,B,C,D) depths and the surface profile became smoother (Figure 3a, 3b). This was confirmed by the measurements of furrows depths before and after the product application (Table I).

DISCUSSION

The results of this work demonstrate a technique which assures a good location of the surface to study (Figures 2a, 2b). There was a replication of the same surface area after 30 days of a cosmetic application which proves the accuracy of the localization method. Our study was carried out on skin surface negative replicas which assured an economy of time and money. When profilometry, surfometry and scanning electron microscopy are employed for determining skin

microtopography, skin positive replicas are necessary. The surface analysis with the CLSM gives the possibility to have an image of the skin microtopography associated simultaneously with measurements of furrows depths and plateaus widths (Figure 3). This apparatus has a remarkable advantage compared to the scanning electron microscope which gives skin surface images of very good quality, but unfortunately, without measurements.

From the present results, it is clear that the control of a cosmetic efficiency can be performed perfectly by the CLSM. The moisturizer employed in this work showed its efficacy by decreasing the depths of furrows (Table I). A noticeable difference could be observed between the profiles of surfaces before and after the product application (Figure 3a, 3b, Table I). The skin surface became smoother.

It is important to mention that there are some essential aspects to consider :

- The surface area studied was not very large (3-4 plateaus). Thus, it is advisable to increase the surface analyzed by the CLSM.
- The exact location of the area (same plateaus, same furrows) is absolutely necessary, otherwise the visualization and the assessment of another area have no interest and give false results.
- It is difficult to compare the results of this work with our previous studies in this field (Makki et al., 1979 ; 1987), unless the same quantitative parameters (R_{tm} : average of furrows depths ; S_m : average of plateaus widths) were employed. For this reason, it is necessary to exert in this technique, the same parameters of profilometry and surfometry. Then, we can be able to compare the different methods and to use the most sensitive one.

Table I Furrows depths measurements
before and after 30 days of a daily cosmetic (C) application

Furrows (10 measurements)	Furrows depth (μm)	
	D0 ($\bar{x} \pm \text{S.D.}$)	D30 (S.D.) ($\bar{x} \pm \text{S.D.}$)
A	80 ± 5	30 ± 1
B	60 ± 3	10 ± 1
C	40 ± 2	10 ± 1
D	80 ± 3	50 ± 1

Each furrow was measured 10 times

D0 The day before cosmetic (c) application

D30 After 30 days of the cosmetic (c) daily application

\bar{x} The mean

S.D. Standard deviation

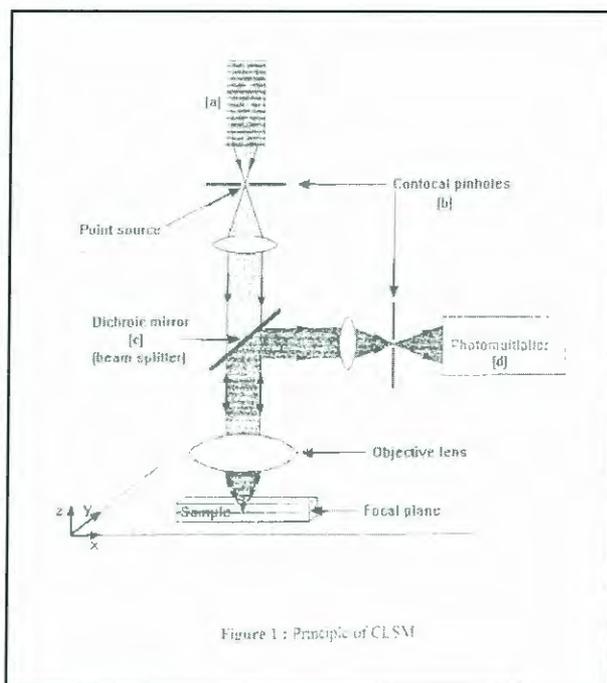
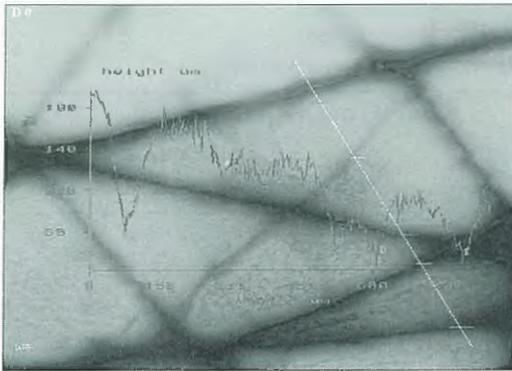


Figure 1: Principle of CLSM

Figure 1

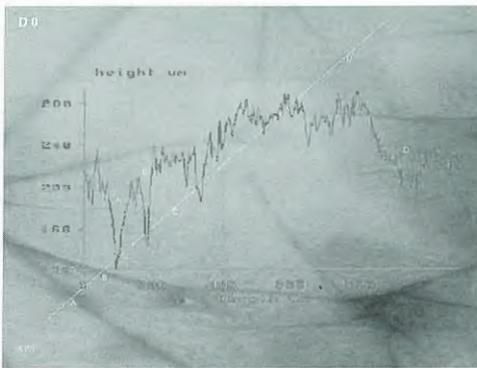


Forearm skin surface at day 0

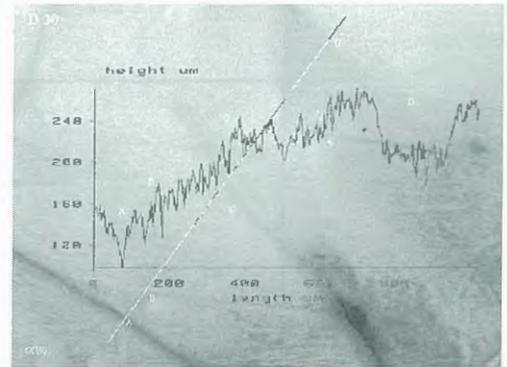


The same skin surface after 5 weeks

Figure 2 Skin surface localised the first day (D0) and 5 weeks after (5W)



Wrinkles near the eye before a cosmetic application



The same skin surface 30 days after the cosmetic application

Figure 3 Wrinkles near to the eye before and after application of a cosmetic (c)

- 3a Before treatment (D0) : the surface profile is showing different incidents and furrows are noticeable.
- 3b The same eye wrinkles after 30 days of the daily cosmetic (c) application : the skin surface profile becomes smoother and furrows are narrower and tight.

References

- 1) **Corcuff P, Lévêque JL.**, *In vivo* vision of the human skin with the tandem scanning microscope. *Dermatology* 1993, **186**, 50-4.
- 2) **Makki S.**, Méthodes d'étude quantitative de la microtopographie de la peau humaine: évaluation de l'efficacité des produits dermo-cosmétiques. *Thèse: Sci. Pharm.*: Besançon: 1987.
- 3) **Makki S, Barbenel JC, Agache P.**, A quantitative method for the assessment of the microtopography of human skin. *Acta Derm Venereol (Stockh)* 1979, **59**, 285-91.
- 4) **Paddock SW, ed.** Confocal microscopy: methods and protocols. Totowa, Humana Press, 1999.
- 5) **Rajadhyaksha M, Grossman M, Esterowitz D, Webb RH, Anderson R.**, *In vivo* confocal scanning laser microscopy of human skin : melanin provides strong contrast. *J Invest Dermatol* 1995, **104**, 946-52.
- 6) **Sheppard CJR, Shotton DM.**, Confocal laser scanning microscopy. Oxford, *BIOS Scientific Publishers*, 1997.

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