

## REPLICA INFORMATION PACKET

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- BioNET protocol for Countour Analysis using Large Area Locators
- Storage and shipping of Replicas

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## Hints for making better skin replicas.

### **SKIN SURFACE PREPARATION:**

All body regions except sebum-rich regions (face, upper back and upper breast):

The less skin manipulation prior to obtaining the replica, the better. Patients should be advised not to wash on the day when the replica is obtained. Application of any topical therapy, including moisturizers should be stopped one week prior to taking the replica, except in cases where replicas are used to evaluate the effect of these substances on the skin. The area should not have been shaven for at least 5 days.

Face and other sebum-rich areas (upper- back, breast): Additionally to the above mentioned, 5-10 minutes prior to taking the replica, the skin may be wiped briefly and gently with a cotton swab soaked in ethanol. This procedure is necessary in order to remove excessive sebum, which especially in oily patients can mask the glyptic lines and alter the surface pattern. Otherwise variations in sebum output may be reflected as artifacts in the replicas.

Moisturizers (O/W or W/O), oily body lotions, occlusive cosmetic preparations, make up etc should not be used on the skin areas to be tested in the last 12 hours before replica is taken. Unless-otherwise mentioned in protocols no topical medication should be allowed that may interfere with the study results. If some cleaning is necessary, cleaning water containing lotion non-alcoholic with non-ionic detergent should be used to remove scales and excess horny layer. After this treatment allow a minimum of 3 hours for recovery of the skin before replica is taken. **The skin must be clean and dry.**

### **Removal of hair:**

**A:** Removal of hair from skin area prior to replica taking: Two methods have been suggested:

1. The area can be cautiously shaven with a razorblade 5 days prior to obtaining the replica.
2. Directly before taking the sample, hairs can be clipped with scissors, avoid touching the surface as much as possible.

**B:** Removal of hair from replica:

The replica is placed over night in 5% KOH-solution, cleaned with a fine brush and washed in de-mineralized water.

### **Skin with visible scales:**

Remove the scales prior to taking the sample, as they may mask the glyptic lines of the skin. Large scales should first be removed with forceps. Smaller scales should be removed with repeated stripping with adhesive tape until all visible scales are removed. It is however a highly subjective procedure. It works well for most psoriatic lesions and in skin with xerosis vulgaris. Thick hyperkeratotic scales, like in ichthyosis vulgaris, are more difficult to deal with.

## **STANDARD POSITIONING OF THE BODY:**

Patients should lie flat so that the area, where a replica will be taken, is at a horizontal position.

**Lying on the back:** The forearms should be placed with their dorsal side upwards. In this position replicas of the following body parts can be taken:

**Face:** It is important that the patient lies relaxed with eyes closed and avoids contractions of mimic muscles.

**Neck:** The neck should be straight. Replicas can also be taken in maximal dorsal extension (neck bent backwards), as this is a very well reproducible position and facilitates the taking of sample.

Dorsal forearm

Back of the hand

Upper arm extensor- side

Breast and abdomen

Upper leg, ventral side

Lower leg, Ventral and lateral side

Dorsum of the foot

### **Lying face down:**

Volar arm

Upper inner arm

Back of the neck

Back

Thigh

Upper leg, dorsal side

Calf

Palms

Soles

### **Environmental factors:**

All measurements should preferably take place in an air-conditioned room with constant temperature and humidity. Patient should rest at least 15 minutes before taking the sample.

**REPLICA PREPARATION:** The following will deal only with replicas made from Silflo. It seems by far the most used material. Its reflectivity is good and the reproducibility of the replicas (surface roughness parameters) is also better than other materials tested.

In principle, making a negative skin replica with this material is easy! On a clean tray or glass plate the silicon rubber material is mixed with a catalyst in a ratio of about 1 drop of catalyst/ml paste. A tray with a round bottom is recommended for best mixing. The two ingredients are mixed carefully together for 15-20 seconds and are finally smeared over the skin area. For a single CuDerm replica-locating ring about 1-2 ml mixture is plenty, corresponding to a final thickness of 2-3 mm.

### **Possible mistakes:**

If catalyst and paste are mixed too vigorously, air bubbles may accumulate in the replica. To correct this problem, apply a very thin layer initially. Follow with a second layer on the back of the original layer in order to strengthen and ensure a flatter base to the replica, and thus ensuring that all points within an area are within the working distance of the analyzer.

The polymerization process is difficult to control. Prior to application the viscosity of the mixture of Silflo and catalyst should be “freely flowing”. A standard polymerization time of 5 minutes has been recommended, but ambient temperature, skin temperature and humidity should be taken into consideration. The plasticity of the residual left on the mixing plate should be a good indication of the state of polymerization.

Sometimes the surface mixture may appear “glossy” or have glossy patches as opposed to the normal non-glossy surface. We have at present no good explanation for this phenomenon, which causes abrupt changes in surface reflection and interferes with optical measuring systems. It may be due to a sub-optimal mixing of Silflo and catalyst or to variations in humidity.

### **DEFINING SITE AND ORIENTATION OF REPLICA:**

When making a replica it is necessary to have a clear indication of North-South and East-West orientation of the anatomical area. On the replica ring the orientation may be indicated with a pencil.

## **Silicone Impression Material**

**SILFLO** is a light bodied dental material with sufficient body to record sub-gingival areas. It flows readily into undercuts and on removal returns to original shape.

### **MIXING**

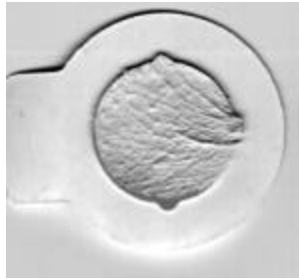
Mix thoroughly on glass slab kept exclusively for this purpose. 1 inch of resin ( $\cong$  1ml) to 1 drop of catalyst. Extra catalyst hastens setting. It is advantageous therefore with larger impressions, using six or more inches of paste, to reduce the number of drops of catalyst. Thus extra time is obtained for mixing. Normal mixing time is one minute.

**Always allow *at least* four minutes setting time.**

**CATALYST: Shake bottle before use. Catalyst evaporates, so always replace cap immediately. Keep cool and out of direct sunlight. If slow setting, use extra catalyst. Catalyst available separately.**

# REPLICA QUALITY EDUCATION

A good quality replica will look like this:



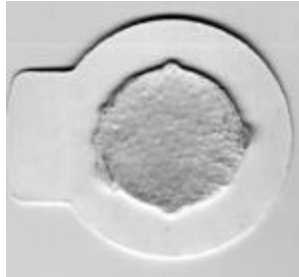
SKIN side



TOP side

The skin side reveals fine detail with coverage of the entire circular area. The top side is a smooth glossy surface. The label area is free of replica paste.

## ***Examples of poor quality replicas:***



Skin oil and makeup reduce the detail in the replica. Skin should be cleaned of surface oil and makeup prior to application.



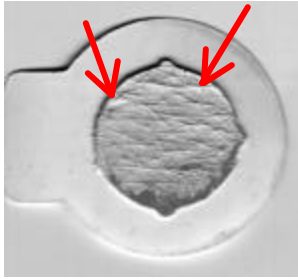
Spots at the arrows are actually pits caused by air bubbles resulting from applying too thick a layer of replica paste initially. Better to apply a very thin layer first then follow up with a thicker layer a few minutes later. This can also be a sign that the paste was beginning to set up at the time it was applied.



Pits and lack of gloss on the top side of this replica indicates the replica paste and catalyst was not mixed well.



Here the paste is not only stringy indicating the resin was starting to harden during application, but the paste is covering part of the tab making the identification of the sample uncertain.



In this example, the glossy spots on the underside (arrows) indicate insufficient mixing of the paste with the catalyst. The dark area at the bottom of the area is an artifact caused by the image lighting system.

Prepared by David L. Miller, Ph. D.

**METHODS**

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**Equipment**

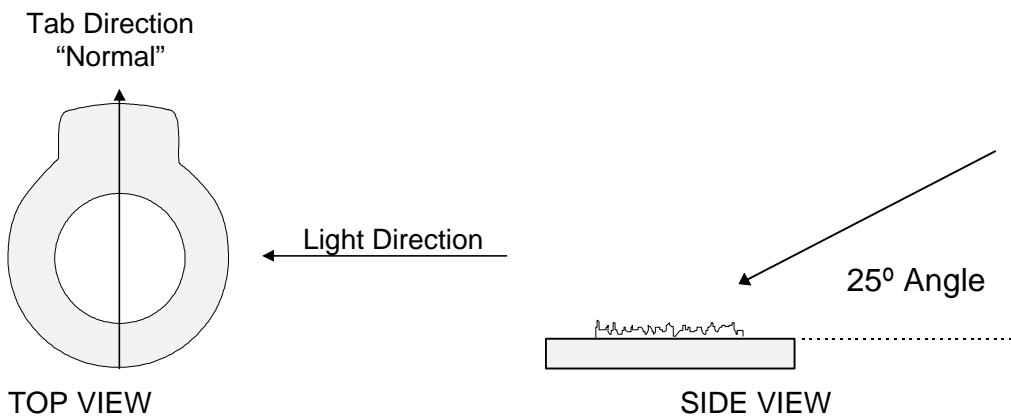
PC: IBM compatible Pentium III with math co-processor and 128 mb memory running under Windows98.

Video: Cohu solid state B&W camera, 50mm lens/30mm extension, ITI OFG frame grabber, SONY Trinitron monitor.

Software: OPTIMAS v6.2, Microsoft EXCEL 2000, StatSoft STATISTICA 6.0

**Sample:** 19mm diameter silicone resin negative replica of skin surface.

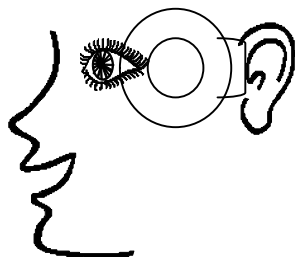
**Lighting:** Collimated light source directed at a 25° angle from the plane of the replica. The replica is placed in a holder that fixes the direction of the tab position of the replica so that the replica can be rotated to align the tab direction normal or parallel to the incident light direction.



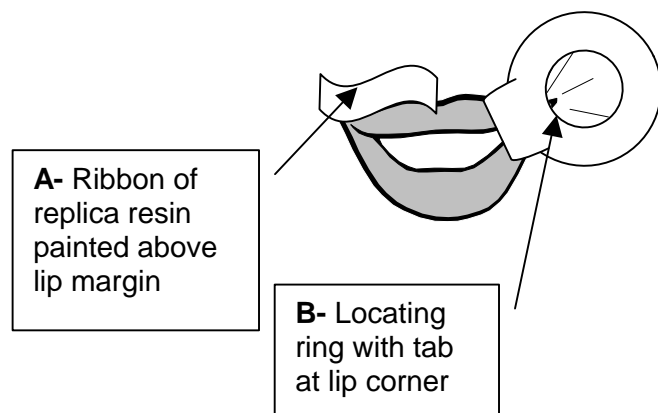
Recommended standard placements:

**Periorbital:**

Locating ring with tab pointing to ear



**Perioral:**



## Replica Analysis

The general background gradient of light intensity is adjusted by applying a 2nd order correction in the direction of the light propagation. The shadow texture produced by the oblique lighting of the negative replica is analyzed by two types of assay methods:

**A.** Measuring the luminance along a set of 10 equal length parallel lines (passes) running across the replica parallel to the lighting direction. The variations in luminance (lightness/darkness) are treated as indicative of the roughness and analyzed by traditional surface roughness statistics:

**Rz-** the average maximum difference in luminance value for five equal length segments in each of the 10 lines traversing the sample. The maximal optical roughness.

**Ra-** the average deviation of the luminance curve about the mean luminance for the same 10 lines. The average optical roughness.

The "R" parameters are reported in the units of brightness (Gray Levels) ranging from 0 to 255.

**FSpace-** distance between markers placed on the lines at luminance changes indicative of fine line spacing (mm).

**Fnum-** number of fine line markers (per mm).

**B.** The replica image area is divided into 10 equal width bands or sub-areas. Shadow like features are detected in each of these bands according to their luminance values being less than the detection threshold<sup>1</sup>. Four parameters are determined from the detected features.

**Spacing-** the mean distance in millimeters between adjacent detected features (i.e. spacing between the midpoints of adjacent shadowy features).

**Breadth-** the average breadth in millimeters of the detected features in millimeters. This parameter is proportional to the depth of the wrinkle producing the shadow.

**Shadows-** percent of the sampled replica area with luminance values less than the detection threshold. This is the relative area of shadows cast by the wrinkles and fine lines in the replica.

**Num Wrinkles-** the total number of features detected in the 10 bands or sub-areas used to calculate spacing and breadth.

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<sup>1</sup> Threshold Algorithm:

CUTOFF = that gray level halfway between black and the most probable gray value in the image. The most probable gray value typically characterizes the flat, featureless regions of the replica.

Shadows =  $100.0 * \text{sum}(\text{ArROIHistogram}[\text{Lo..}(\text{CUTOFF} + 1)]) / \text{totalpixels}$ .

## INTERPRETATION GUIDELINES

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The 8 wrinkle texture parameters reported in our analysis measure various aspects of the image produced by the replica surface. Generally if there is a substantial smoothing effect, there will be consistent significant changes in several parameters.

**Rz** and **Ra**, optical counterparts of classic “stylus” roughness texture parameters: *increase with increasing roughness*. The diagrams below illustrate the definitions. The profile in the diagram is the brightness profile generated by the angled lighting of the wrinkles on the replica. Note that the amplitude of the profile is not proportional to the depth of the wrinkle but represents the intensity of the *shadows* behind the wrinkles and *highlights* in front of the wrinkles. **Rz**- the maximum difference in luminance value (measured at five equal length segments traversing the sample). **Ra**- the average deviation of the luminance curve about the mean luminance.



**Fspace**, *distance* between markers in mm indicative of fine and coarse lines. In the diagrams above these would be placed at each maximum in the luminance profile.

**FNum**- *number* markers per mm. As lines and creases disappear, Fspace increases and FNum decreases.

**Spacing**- the mean distance in millimeters between adjacent strong shadow features. Decreases with conversion of deep wrinkles to fine wrinkles (Moisturization). Increases with disappearance of wrinkles.

**Breadth**- proportional to the depth of the wrinkle producing the shadow. May or may not change. Decreases as wrinkles become *shallow*. Not sensitive to the number or length of wrinkles.

**Shadows**- This is the relative *area* of shadows cast by all the wrinkles and fine lines in the replica. It is sensitive to both the length and depth of the wrinkles. Decreases with smoothing of the skin.

**Num Wrinkles**- the total number of shadowy features available to calculate spacing and breadth. Generally decreases with smoothing of the skin (fewer visible features).

## BIBLIOGRAPHY

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Profilometry of skin- A useful tool for the substantiation of cosmetic efficacy, Cook, J Soc Cosmet Chem 1980; 31:339-359.

Optical profilometry: An objective method for quantification of facial wrinkles, Grove, Grove, and Leyden. J AM ACAD DERMATOL 1989;21:631-7.

Treatment of photodamaged facial skin with topical tretinoin, Leyden et al. J AM ACAD DERMATOL 1989;21:638-44.

Roughness (measured by profilometry: mechanical, optical, and laser), Müller, in "Bioengineering and the Skin: Methods and Instrumentation" Berardesca et al, Eds. CRC Press, Boca Raton, 1995.

Review articles from "Handbook of Non-Invasive Methods and the Skin", Serup and Jemec, Eds. CRC Press, Boca Raton, 1995.

Stylus method for skin surface contour measurement, Gassmuller et al p83.

Skin surface replica image analysis of furrows and wrinkles, Corcuff and Leveque, p89.

Laser Profilometry, Elfsen et al, p97

Three dimensional evaluation of skin surface: micro and macro-relief, Mignot, p107.