On in-vivo skin topography metrology and replication techniques

B.-G. Rosén1,2, L. Blunt3, T. R. Thomas1,4

1Functional Surfaces Research Group, Halmstad University, SE-439 31 Halmstad, Sweden,
2Toponova AB, Halmstad, Sweden
3Centre for Precision technologies, University of Huddersfield, UK
4Avalon Technology, UK

E-mail: bg.rosen@set.hh.se; l.a.blunt@hud.ac.uk; tr.thomas@set.hh.se

Abstract. Human skin metrology is an area of growing interest for many disciplines both in research and for commercial purposes. Changes in the skin topography are an early stage diagnosis tool not only for diseases but also give indication of the response to medical and cosmetic treatment. This paper focuses on the evaluation of in vivo and in vitro methodologies for accurate measurements of skin and outlines the quantitative characterisation of the skin topography. The study shows the applicability of in-vivo skin topography characterisation and also the advantages and limitations compared to conventional replication techniques. Finally, aspects of stripe projection methodology and 3D characterisation are discussed as a background to the proposed methodology in this paper.

1. Introduction
Analysis of the status of human skin at different times, locations and at different scales provides an objective evaluation of factors like, ageing, and medical status as well as effectiveness of cosmetic treatments [1,2]. Important skin features like roughness or topography along with tactile properties, e.g. friction and elasticity against a finger, are here important functional features to quantify for dermalogists and cosmetologists. Corcuff and Pierard [2] classify skin imaging according to their invasiveness and the thickness of the tissue removed for the analysis. Tape stripping can be used for the analysis of consecutive stratum corneum layers while biopsy or shave biopsy provides skin layers down to the hypodermis or in the latter case the papillary dermis. Microscopy- or confocal techniques can be used to represent the samples given by those invasive- or semi invasive methods. Indirect methods are based on analysis of replicas from the “living skin”. Here, 3D surface topography methods like confocal microscopy and 3D stylus- and white-light interferometry measurements give deep insight of the detailed 3D features of the replica for further quantitative parametric evaluation, e.g. wrinkle depths, number of pores, mean amplitude and mean wavelengths. In-vivo measurements are grouped as non-invasive methods [2] and here the naked eye observations, photography and video-microscopes have been complemented lately with the fast non-contact 3D topography system used in this study.
The concept of a “living” substrate—the human skin, measured by vibration sensitive 2D- and 3D-measuring devices like white-light scanning interferometers or traditional stylus techniques has in practice been impossible and this has limited the process to measurements of “dead” polymer replicas of the skin. There are several inherent disadvantages with this indirect technique such as, possible uncertainties regarding the accuracy of the replication, problems of exposing highly sensitive and/or damaged skin to the polymer materials and the “peeling off” forces. Direct non contact techniques would clearly have none of these problems, yet there still is some conjecture as to their usefulness.

In this paper the use of a 3D optical stripe projection method has been investigated both on the real skin and the replicas of corresponding areas. Rohr and Schrader compared the stripe projection method with laser profilometry on replicas [3] and retrieved a high correlation (R=0.923) for maximum peak-to-valley amplitudes. In the present study areas were relocated using manual image recognition techniques. Comparative measurements between the direct and indirect methods have been evaluated using 3D characterisation parameters and areal analysis methodologies.

Traditionally the measurement of skin topography has used static measurements of silicone replicas through the application of contacting stylus or some sort of optical confocal system. The technique has proved to be very useful and can recover the skin topography with excellent accuracy, an example is shown in figure 1 of the topographical data collected from a melanoma present on damaged skin. The figure clearly shows the capability of traditional methods for analysing the size shape and anisotropy of such features.

![Figure 1. Topographical analysis of skin melanoma, data used to analyse size and growth rate](image)

There are a number of drawbacks to the traditional in vitro static replication techniques. Firstly from a clinical point of view sensitive or damaged skin responds poorly to the application of replicants Secondly from a measurement point of view traditional techniques take time to collect the data which precludes them from real time use at clinic and traditional techniques measure only limited areas which are often smaller than the area under clinical investigation [4] (wrinkles, scars or stretch marks).

In vivo skin measurement based on optical fringe projection has opened up the possibility of direct measurement of the topography of “living” skin [5] This paper seeks to validate the method by comparing the measurements of living skin, measurements of the same area based on replicas and comparing the results to conventional static replication methods.

2. Materials and Methods

The fringe projection apparatus—GFM PRIMOS ¹ used to measure both real skin- as well as replica surfaces is based on triangulation using temporal phase shifting techniques [5]. Parallel stripes are projected onto the measuring object and the deviation caused by the objects shape and topography from projected perfectly parallel lines are captured by a CCD camera and transformed into X,Y,Z-coordinates with a lateral resolution (29.1*30.1um) given by the measurement area (18.369*14.441mm) and number of pixels in the CCD camera picture (632*479). The vertical

¹ GFMesstechnik GmbH, Teltow, Germany, http://www.gfmesstechnik.de
resolution is 1.84µm (lateral x-length/10000). PRIMOS needs four images to produce a full 3D image and the shooting time of 68µs per 3D image was possible due to the 60Hz CCD camera system used.

Wavelength content was analysed using Omisurv v. 1.67. The replica material used was the SILFLO 3 silicone and two in-vivo measurement areas and replicas at different locations where measured on the forearm of the 43 year old test patient. The skin was shaved and peeled with transparent adhesive tape to remove the top most stratum corneum layers. Pure ethanol finished the preparation of the skin.

Figure 2. (a) In vivo fringe measurement of skin. (b) Optical photograph of measured zone (c) optical photograph of replica.

To enable accurate relocation of measurements made in-vivo and with indirect replica measurement the features inherent in 3D surface metrology were utilized. Visual relocation of areas was accomplished by using 3 glued steel balls of 4mm diameter, similar to the technique developed by Blunt et. al [6], and Wenneberg et al. [7]. It is possible to achieve good relocation using this method, total error per axis of ≤ two pixels (60µm).

3. Results
In order to obtain useful results all the data sets were filtered using a robust Gaussian filter to remove form error. The 3D parameter [8] values for two of the surfaces an original in vivo and a replica measurement are given in table 1

Table 1. An example of parameter values for in vivo skin measurement and its relocated replica

| 3D Parameters                  | Replica | Original | Replica zoom | Original zoom |
|--------------------------------|---------|----------|--------------|---------------|
| Sa/um (average roughness)      | 17.0    | 15.2     | 16.6         | 12.5          |
| Sz/um (max P-V roughness)      | 171.7   | 173.2    | 117.3        | 83.17         |
| Sq/um (RMS roughness)          | 21.2    | 19.4     | 20.6         | 15.7          |
| Ssk (skewness)                 | -0.151  | 0.001    | -0.56        | -0.238        |
| Sdq (surface slope)            | 0.208   | 0.221    | 0.229        | 0.207         |
| Sds(1/mm²) (peak density)      | 5.63    | 9.42     | 7.79         | 9.73          |

Figure 3 shows “large area measurements” taken on both the “real” and replica surfaces. Specific features were then zoomed for higher scale analysis of the two data sets, figure 3(c) and (d).

2 Digital Metrology Solutions Inc.; USA; http://www.digitalmetrology.com.
3 Flexico developments, Davis Healthcare Services Ltd.; Summit Road; Potters Bar; Herts EN6 3EE; England.
Figure 3. Large area measurements of (a) Actual skin measured in vivo (b) replica of same area skin and zoomed area of easily identifiable features skin (c) and replica (d)

Following the area analysis, relocated profiles were extracted from the data sets collected and compared for wavelength content. An example of two such profiles is shown in figure 4.

Figure 4. Wavelength analysis of two extracted relocated profiles from the large areas in fig. 3. Wavelengths (x-axis) in mm are plotted against corresponding amplitudes in um (y-axis). Wavelengths above 0.25mm display a corresponding behaviour between replica and original (in-vivo) measurement.
4. Discussion and Conclusions

It is a well established fact that the fringe projection methods can capture data from soft polymer surfaces consequently the authors have used the replica method as a base line from which to judge the effectiveness of the fringe methods when applied to “living” skin. It is clear from visual perception of the results that the fringe projection accurately captures the main topographical elements of “living skin”. Analysis of the numerical parameters however, shows that the replica technique consistently give higher values for the amplitude roughness parameters. It is considered that the difference in roughness amplitude values result from factors related to the replication technique as such (elastic effects, viscosity properties, optical color and reflectance differences, along with selection of sampling distance together with the inability of the fringe technique to penetrate deep valley features on living skin. In contrast when these features are captured as peaks on the replica the fringe technique successfully records them and more research is needed to clarify the matters raised here. When considering the spacing parameter Sds, this indicated that the original data measured contained more high frequency/short wavelength features. To investigate these differences further, extracted profiles were examined for their wavelength content an example is given in Figure 4. The analysis showed that the fringe technique in combination with the replication failed to record wavelengths close to and below 0.25mm. This inability to distinguish fine scale features is clear in figure 3c and 3d where it is clear the original in vivo measurement has distinguished finer scale features in the topography.

Overall direct in vivo skin measurement appears to be feasible for recording relative changes in skin topography in response to medication or as a measure of the progress of skin diseases. It is fast and can be carried out within a clinical environment with minimal patient inconvenience. However analysis of deep pitted skin features is somewhat limited and would require replication and further measurement techniques.

References

[1] Asserin J., Zahouani H., Humbert Ph. Couturaud V., Mougin D.; Measurement of the friction coefficient of the human skin in vivo Quantification of the cutaneous smoothness; Colloids and Surfaces B: Biointerfaces 19; pp 1-12; (2000).
[2] Jacobi U., Chen M., Frankowski G., Sinkgraven R., Hund M., Rzany B., Sterry W., Lademann J.; In vivo determination of skin surface topography using an optical 3D device; Skin Research and Technology, Vol. 10, pp. 207-214; (2004).
[3] Corcuff P., Piéard G. E.; Skin imaging: State of the art at the dawn of year 2000; in Elsner P., Barel A.O., Berardesca E., Gabard B., Serup J. (eds): Skin Bioeng. Techniques and Apps. in Dermatology and Cosmetology; Vol. 26, Cupde: pp1-11; Karger Publishers; Basel; (1998).
[4] Rohr M., Schrader K.; Fast Optical in vivo Topometry of Human Skin (FOITS); SÖFW Journal; vol. 124; pp. 52-59; (1994).
[5] K.J. Stout and L.Blunt, Nanometers to Microns 3D Surface Measurement in Bio-Engineering, Proc. 2nd Int. Conf. on Surface Eng. Adelaide Australia March (1994).
[6] Frankowski G., Chen M., Huth T.; Real-3D Shape Mesurement with Digital Stripe Projection by Texas Instrument Micromirror Devices DMD; Three-Dimensional Image Capture and Aplications III, SPIE, Vol. 3958; pp. 90-105; (2000).
[7] Blunt L., Ohlsson R., Rosén B.-G., A Comprehensive Study of 3D Surface Topography Measuring Instruments, In: Hedenqvist P., Hogmark S. & Jacobson S. (ed), Proceedings of the 6th Nordic Symposium on Tribology NORDTRIB 94, Uppsala, Sweden, June 12-15, (1994).
[8] Wennemerberg A., R. Ohlsson, B.-G Rosén, B. Andersson, Characterizing 3D Topography of Engineering and Biomaterial Surfaces by Confocal Laser Scanning and Stylus Techniques, Medical Engineering and Physics, Vol.18, No.7, pp. 548-556, (1997).
[9] Blunt, L., Jiang, X. (eds.); Advanced Techniques for Assessment of Surface Topography – Development of a Basis for 3D Surface Texture Standards “SURFSTAND”; Kogan Page Science; London and Sterling VA; ISBN 1-9039-9611-2; (2003).