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Ezerskaia, A.; Pereira, S. F.; Urbach, H. P.; Varghese, Babu

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Quantification of changes in skin hydration and sebum after tape stripping using Infrared spectroscopy

A. Ezerskaia^{a,b}, S. F. Pereira^b, H. P. Urbach^b, B. Varghese^a

^a Personal Care and Wellness Department, Philips Research, 5656AE, Eindhoven, The Netherlands

^b Optics Research Group, Delft University of Technology, 2628 CH, Delft, The Netherlands

ABSTRACT

Skin barrier function relies on well balanced water and lipid system of stratum corneum. Optimal hydration and oiliness levels are indicators of skin health and integrity. We demonstrate an accurate and sensitive depth profiling of stratum corneum sebum and hydration levels using short wave infrared spectroscopy in the spectral range around 1720 nm. We demonstrate that short wave infrared spectroscopic technique combined with tape stripping can provide more-quantitative and more reliable skin barrier function information in the low hydration regime, compared to conventional biophysical methods.

Keywords: Hydration, lipids, tape stripping, infrared spectroscopy

1. INTRODUCTION

The stratum corneum is the outermost layer of the epidermis and is composed of the corneocytes and an intercellular lipid bilayer matrix. The hydration and sebum retaining ability of the skin is primarily related to the stratum corneum [1]. Optimal skin lipids and water levels are reported as essential indicators of skin integrity and functionality, whereas disrupted balance is found in different dermatological disorders such as psoriasis, atopic eczema, edema, rosacea, melisma [2-4].

Oily skin look bright, flaxy and turgid, pale yellow. Dry skin is rough, stiff, fragile, opaque, pale gray [5]. The physiological physical properties of skin are also highly dependent on the skin moisture content. Epithelium remains flexible when it contains 10-20% water, but becomes brittle, when it drops below 10% [6]. Nevertheless, while moist is directly affecting properties of skin, the capacity of skin to proceed water exchange and keep certain amount depends on several factors. Among them superficial lipid in cope with sebum and intercellular lipids play the key role. At normal physiological temperatures (approx. 20-40 °C) these carbon chains may be in a liquid state [7]. Double layers of such lipids in the liquid crystalline state will allow water to pass through the membrane more or less freely [8] and a cell membrane is therefore not a barrier to water. On the other hand, bilayers in the close packed crystalline (gel) state will effectively prevent the penetration of water molecules [9]. The composition of the barrier lipids makes it obvious that the majority of the stratum corneum lipids will form crystal-(gel-) structures, hence provide a water tight enclosure. The transmission electron microscopy demonstrates that the inter corneocyte bilayers are stacked in multiple layers [10] and such mosaics of domains will constitute an essentially water tight structure. Any water molecule escaping from the body will have to suffer a meandering way out of the system. A random diffusion path in the inter-bilayer water sheath will occupy a comparatively long time compared to the vertical passage through a liquid crystalline area to reach the water sheath separating the past bilayer from the next one.

Hydration rises within skin from outmost layer of stratum corneum towards layer of living cells from ~30 % to ~70% in the normal skin [11-13]. Where hydration of outmost layers can vary in the range 25 – 50 % dependently on skin type; in extreme cases the hydration of outmost layer for dry skin can rich up to 10 % [14-15]. Stratum corneum can be described by simplified model of “brick and mortar”, where coenocytes (bricks) are embedded in the continuous matrix of specialized lipids (mortar). Stratum corneum consist of approximately 20 volume % of lipids (or about 15 % of the dry weight) [3, 16-17].

The efficacy of the barrier function must be correlated with physical parameters such as skin humidity and oiliness. Tape stripping is well established method for investigating skin barrier function, quantify the penetration of drugs and to evaluate different skin disorders [18-19]. Histologically, though, the SC is a non-uniform, inhomogeneous membrane, but distribution of the barrier function remains uncertain. Quantification of the stratum corneum hydration and lipids distribution with high resolution provides better understanding of the protective properties of the epidermis. The desired high resolution of the profiles is limited to one layer of stratum corneum due to structure. Tape stripping is well established method for investigating skin barrier function, quantify the penetration of drugs and to evaluate different skin disorders [20-21]. Thereby we employ the most suitable investigation method – tape stripping along with infra-red spectroscopy and conventional measurement techniques.

Many biophysical methods have been reported for measuring skin hydration and sebum levels independently. However, no non-contact devices and methods have been reported for quantitative spatial mapping of these components simultaneously. Recently we demonstrated the feasibility of a non-invasive short wave infrared spectroscopic technique for simultaneous measurement of oiliness and hydration levels of the skin [20]. The method is based on differential detection in the spectral region around 1720 nm based on the optimal wavelengths corresponding to the lipid vibrational bands that lay “in between” the prominent water absorption bands.

The aim of this study is to quantify the depth resolved changes in skin hydration and sebum levels after tape stripping using the infrared spectroscopic set-up and compare the results with conventional devices such as Corneometer, Sebumeter and AquaFlux TEWL instrument.

2. MATERIALS AND METHODS

The experimental setup (fig. 1) used for the skin hydration and oiliness level measurement comprises three quasi continuous laser sources, beam shaping optics and mirrors to guide the laser beam via the beam path. The laser sources (LD1, LD2, LD3) were short wave infrared semiconductor lasers diodes emitting a wavelength of 1720 ± 4 nm, 1750 ± 5 nm, 1770 ± 20 nm emitting approximately 40 mW at each wavelength. The output of laser sources are spatially combined along the same optical path using flipping mirrors (FM1, FM2), beam shaping optics, polarizers (P1s, P2p) and the beam is focused through a central aperture in the mirror (M5) before it illuminates an area of approximately 12.6 mm² on the skin surface with a power of approximately 20 mW for each wavelength. Light backscattered from the skin was collimated and reflected by the mirror (M5) and is focused to the detector (PD) using a focusing lens (L10). The wavelengths 1720 nm and 1750 nm are used for estimating the sebum content and 1750 nm and 1770 nm for the water content. Modified Beer's law was used for quantitative estimation of the level of hydration and lipids.

Measurements were performed with the experimental set-up, sebumeter, corneometer, AquaFlux TEWL instrument after each tape stripping. Every measurement was repeated five times with each measurement device. Numerous factors can affect outcome of the tape stripping as it depends on pressure of the applicator and duration of application, speed of the stripping, anatomic side, temperature, time of the day, sex and age, number of layers of stratum corneum, it's thickness, amount of lipids, skin furrows and material of the tape [22-23]. For our study we used large D-Squame Sampling Disc with the diameter 30.16 mm. The controlled pressure was applied on the tape by D-Squame Pressure Instrument (pressure applied: 225 gr/cm²) for 10 seconds. Tapes were removed by one fast movement with D-Squame angular tweezers to ensure minimal variation in the conditions of tape stripping, thereby variation in amount of removed from the skin material remains minimal.

The study was held on a female volunteer (26 years) with no history of atopic dermatitis, asthma, allergy, contact dermatitis, or any other skin disorders. The hydration and lipids profile was measured in the area of forehead (T-zone). Forehead represents a skin area with moderated density of sebaceous glands in the skin along with medium level of hydration in the facial skin [24-25]. All measurements were performed in climate controlled room with temperature 21 ± 1 °C, 45 ± 5 % humidity. Prior to the experiment participant had acclimatization for 30 minutes.

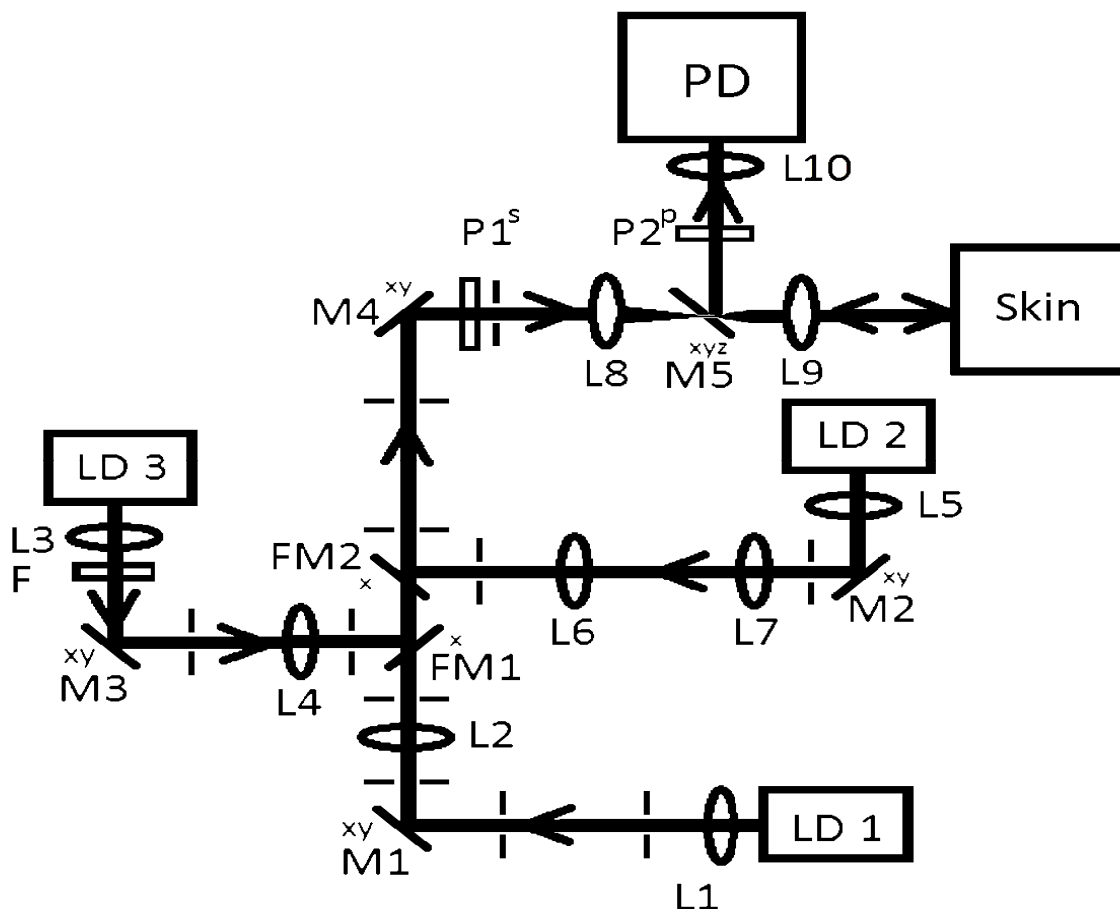


Figure 1. Schematic of the experimental set-up: LD1 – Laser Diode (1720 ± 4 nm, Roithner Laser), LD2 – Laser Diode (1750 ± 5 nm, Roithner Laser), LD3 – Laser Diode (1770 ± 20 nm, Roithner Laser), F – Narrowband filter (1770 ± 5 nm, Spectrogon), M1, M2, M3, M4 – mirrors (ThorLabs), M5 – Mirror with a central aperture, FM1, FM2 – Flipping mirrors (ThorLabs) L1, L3, L5 – Aspheric lenses (ThorLabs), L2 ($f = 300$, ThorLabs), L4 ($f = 300$, ThorLabs), L6 ($f = 75$, ThorLabs), L7 ($f = 150$, ThorLabs), L8 ($f = 35$, ThorLabs)– Plano convex lenses L9 ($f = 35$, ThorLabs) – Biconvex lens, L10 ($f = 25.4$ mm, LA1951-C) P1s, P2p – polarizers (ThorLabs), PD – photodiode (DET30B/M, ThorLabs).

3. RESULTS AND DISCUSSION

Figure 1 shows of changes in skin hydration and sebum after tape stripping and its comparison to other biophysical devices such as Corneometer, Sebumeter and AquaFlux TEWL instrument. Base line represents rather a thin layer of sebum present on the skin at the time of measurement [24]. This point is usually discarded in tape-stripping studies [21]. The casual sebum level gives contrast to the sebum measurement by sebumeter and makes dramatic drop in the skin superficial sebum level during tape-stripping. Noticeable change in the sebumeter readings can be observed during stripping of superficial layer of stratum corneum (2 tapes) and rather insignificant change up to 8-th tape followed by nearly constant of measured lipids level. Forehead skin has moderate density on sebaceous glands, thus sebum can be absorbed from the shafts of the glands by sebutape after tape stripping on a certain level, which is observed in the measurements. The sebutape consist of porous lipophilic and hydrophobic polymer and the method is more sensitive to the loosely bound sebum than the bound intercellular structure lipids. Therefore sebumeter is not recommended for skin lipid level profiling during tape stripping. The depth of penetration of laser beam in this set-up is about $350 \mu\text{m}$, which is

beyond the thickness of a stratum corneum layer. The spectroscopic method is sensitive to both bound and non-bound lipids and therefore makes it possible to measure the changes in the amount of lipids present in stratum corneum during tape stripping. However the measurements indicate the amount of the lipids left in the stratum corneum, rather than quantity in one or several layers of it, due to depth of penetration. The golden standard for water and lipids profiling remain confocal Raman spectroscopy.

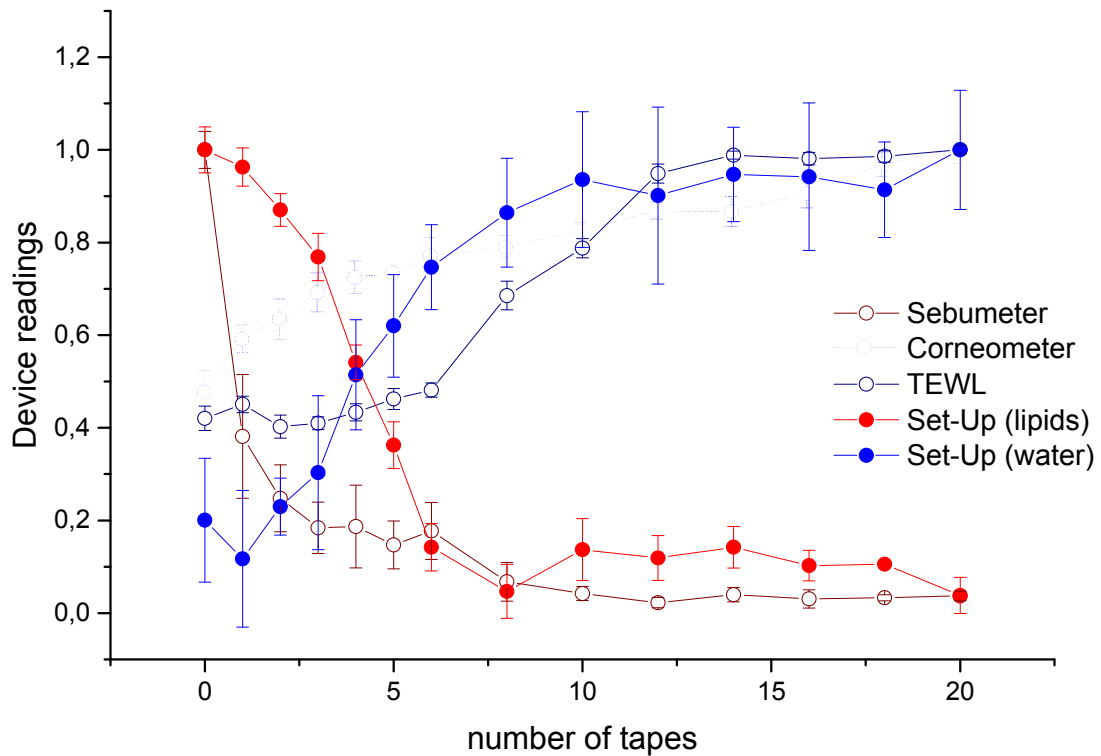


Figure. 2. Hydration and lipids profile obtained on the forehead T-zone) with Infrared spectroscopy and corresponding reference values obtained with Corneometer, AquaFlux TEWL instrument and Sebumeter.

Hydration measurement by Corneometer, AquaFlux TEWL instrument and experimental set-up show increase of water concentration as a function of the depth in the skin. Corneometer appear to record slower changes, than other devices. This device is based on measurements of the skin capacitance, which can be influenced not only by hydration level changes, but also by the amount of lipids level as well. This may cause cross-reading to appear. Naturally while level of water is increasing in the stratum corneum, level of lipids content drops, this way decay of the one component slows down increase of overall reading of the device, showing slower development of the hydration level change in the skin. AquaFlux TEWL instrument has different measurement approach and is sensitive to the water evaporated from the skin surface. The devices shows hydration level changes in the skin after tape stripping, rather than the amount of hydration present in the layer of skin that was removed. Additionally, infrared set-up measurements show high sensitivity in the low hydration regime [26-29]. Previous reports showed good correlation between short wavelength infrared spectroscopy and Raman spectroscopy [27-29].

4. CONCLUSIONS

In this report we quantify the depth resolved changes in skin hydration and sebum levels after tape stripping using the short wave infrared spectroscopic set-up and compare the results with conventional devices such as Corneometer, Sebumeter and AquaFlux TEWL instrument. We demonstrate that short wave infrared spectroscopic method in the spectral range around 1720 nm allows accurate and sensitive depth profiling of stratum corneum sebum and hydration levels. The proposed rapid and non-contact method allows qualitative and quantitative estimation of different water and lipid based topically applied substances on the skin. We anticipate that short wave infrared spectroscopic technique combined with tape stripping can provide much more-quantitative and more reliable skin barrier function information in contrast to conventionally employed biophysical methods.

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