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The Skin's Secrets Revealed using RZ-660 an In vivo Raman analyzer

Hichem KICHOU[1,*], Fatima HIBTI[1], Eric TEBOUL[1], David D'Haeyer[1], Artur Aleksanyan[1] and Florian FORMANEK[1].

[1] HORIBA Europe Research Center, 14 Boulevard Thomas Gobert, Pass. Jobin-Yvon, 91120 Palaiseau, France.
* Corresponding author : Hichem KICHOU, Hichem.kichou@horiba.com

1. Introduction

In vivo skin analysis presents unique and complex challenges due to the skin's heterogeneous, dynamic, and multi-layered architecture. The human skin is composed of distinct layers—namely the *stratum corneum*, epidermis, and dermis—each with unique biochemical compositions and functions. This intricate structure, coupled with individual variability arising from factors such as age, ethnicity, hydration level, and environmental exposure, makes standardized assessment particularly difficult [1-2]. Moreover, the need to preserve the skin's structural and functional integrity during analysis limits the application of traditional diagnostic techniques, many of which are invasive or provide insufficient spatial or chemical resolution.

Conventional methods, such as histology or tape stripping, often require skin biopsies or physical disruption of the skin barrier, which restricts their usability in both clinical dermatology and cosmetic science. Imaging techniques like confocal microscopy or optical coherence tomography, while non-invasive, may lack molecular specificity or are limited in their ability to assess certain biochemical parameters [3]. As such, there is a growing demand for analytical tools capable of delivering non-invasive, high-resolution, and reproducible molecular-level data, which is crucial for understanding skin physiology and evaluating the efficacy of topical treatments.

In this context, Raman spectroscopy has gained considerable attention as a powerful, non-destructive analytical technique capable of providing detailed insights into the molecular composition of biological tissues. It enables label-free, depth-resolved analysis of skin components such as water, lipids, proteins, and exogenous compounds, with micrometer-scale spatial resolution [4]. Its ability to detect subtle biochemical variations without the need for sample preparation makes it especially valuable for in vivo applications. This study explores the use of the RZ-660 In Vivo Raman analyzer as a non-invasive tool to assess key skin parameters *in vivo*. Specifically, we investigate the analyzer's capability to characterize hydration levels, lipid content, and the interaction of the skin with topical products. The findings aim to demonstrate the

potential of Raman spectroscopy as a reliable method for both dermatological diagnostics and cosmetic research.

2. Materials and Methods

2.1 Instrumentation

In vivo skin measurements were carried out using the In Vivo Raman Analyzer “RZ-660”. The system is equipped with a 660 nm laser, delivering 18 mW at the skin surface, in compliance with safety guidelines IEC60825-1:2014,Safety of laser products. This wavelength offers a favorable balance between Raman scattering efficiency and minimization of skin autofluorescence.

The optical system includes a high-numerical-aperture (NA 0.85) microscope objective, allowing precise focusing and efficient light collection. The Raman-scattered light is diffracted using a holographic grating and detected by a cooled CCD spectrometer, offering a spectral resolution of ~5 cm^{-1} and an axial (depth) resolution better than 5 µm. This setup enables detailed, depth-resolved molecular analysis of the stratum corneum and upper layers of the epidermis. A schematic representation of the instrumental configuration is provided in Figure 1.

Figure 1. Schematic representation of the instrumental setup, featuring an operator and a volunteer.

2.2 Measurement Protocol

Raman spectra were acquired from the skin surface to a depth of 60 µm (step of 3µm), with 5 seconds integration time per spectrum and 2 accumulations to improve signal-to-noise ratio. These acquisition parameters strike a balance between spectral quality and acquisition time suitable for *in vivo* measurements, minimizing motion artifacts and preserving participant comfort (Profile time < 4 min).

All measurements were conducted on the ventral side of the forearm, a region commonly used for dermatological research due to its accessibility and relatively uniform skin structure.

2.3 Study Population

The study involved five healthy Caucasian volunteers, aged between 25 and 45 years, recruited from our laboratory staff. Before each measurement session, volunteers were acclimated for 20 minutes in a controlled environment (temperature and humidity regulated) to stabilize skin conditions and reduce external variability.

All experimental procedures were non-invasive and conducted in accordance with institutional ethical standards.

2.4 Data Processing

Data acquisition was managed through the instrument's proprietary software. Spectral processing and analysis were performed using LabSpec6 (HORIBA Scientific). Key steps included spectral normalization, baseline correction through polynomial fitting, and multivariate analysis, to extract relevant molecular features and ensure reproducibility. These processing steps allowed for robust interpretation of Raman data and facilitated comparisons across different depths and volunteers.

3. Results

3.1 Depth-Resolved Hydration Profiles

In vivo Raman spectroscopy enabled the generation of depth-resolved water content profiles for all five volunteers (Figure 2a). The resulting curves show a progressive increase in water content from the skin surface through the *stratum corneum* (SC), followed by a plateau that corresponds to the viable epidermis. This pattern is consistent with the known barrier function of the SC, which regulates transepidermal water loss (TEWL).

The hydration gradient also served to determine the SC thickness based on the inflection point of the water profile. Across the five subjects, SC thickness ranged between 9 and 13 µm, with an average value of 11 ± 2 µm, aligning well with previously reported *in vivo* values [5-6].

These hydration profiles are particularly valuable for cosmetic product development and efficacy testing. They enable non-invasive evaluation of hydrating agents *in vivo*, providing a reliable and reproducible way to assess product performance without the need for invasive sampling. Additionally, they allow for real-time monitoring of product penetration and moisture retention, offering insights into both the immediate and sustained effects of topical treatments. Such detailed profiling also supports subject stratification based on skin barrier quality, making it possible to design more targeted and effective testing protocols. Finally, the depth-resolved hydration data can be used to guide formulation strategies, tailoring active ingredient delivery according to the desired penetration depth and hydration objectives.

3.2 Stratum Corneum Thickness Determination

The system’s axial resolution (<5 µm) makes it possible to clearly identify skin layer transitions. SC thickness was determined using the Inflection point in the hydration profile (Figure 2b).

This non-invasive measurement of stratum corneum (SC) thickness is particularly valuable for evaluating the efficacy of exfoliants or cell turnover agents, assessing the permeation potential of formulations across the skin barrier, and understanding the impact of environmental or pathological conditions, such as xerosis or atopic skin, on barrier integrity.

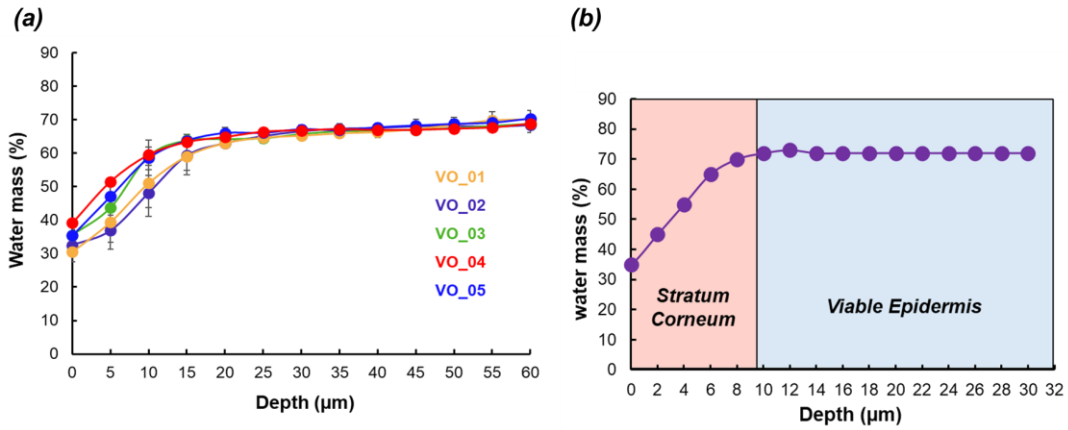

Figure 2. (a) Measured water mass (%) for five volunteers (VO_01 to VO_05); (b) *Stratum corneum* thickness calculation from the water mass profile.

3.3 Lipid Conformation Analysis

Spectral analysis of the CH_2 stretching region (2850–2885 cm^{-1}) allowed for the evaluation of lipid chain conformation in the SC (Figure 3a). Based on peak intensities, a lipid order index was derived for each subject. Narrow, symmetric peaks are indicative of well-ordered (crystalline) lipid chains, while broader, shifted peaks suggest disordered (liquid) structures.

This molecular-level parameter is highly relevant in research and development for assessing the effect of barrier-repairing or lipid-rich formulations, evaluating the mildness or aggressiveness of cleansers and surfactants, and monitoring lipid reorganization in compromised or aged skin. Changes in lipid structure can serve as early markers of barrier dysfunction or restoration, often occurring before visible symptoms appear [7].

3.4 Quantification of Lipid Content

The relative lipid content was calculated from the integrated area under specific Raman lipid bands (Figure 3b). Inter-individual variability was observed, highlighting physiological differences in lipid composition among volunteers. These variations offer insight into barrier status and skin type classification (e.g., dry vs. oily skin) [7].

The impact for product formulation includes supporting tailored formulations based on individual lipid levels, evaluating the ability of products to stimulate endogenous lipid synthesis, such as ceramides and fatty acids, and optimizing seasonal or climate-adaptive product lines based on environmentally influenced lipid depletion .

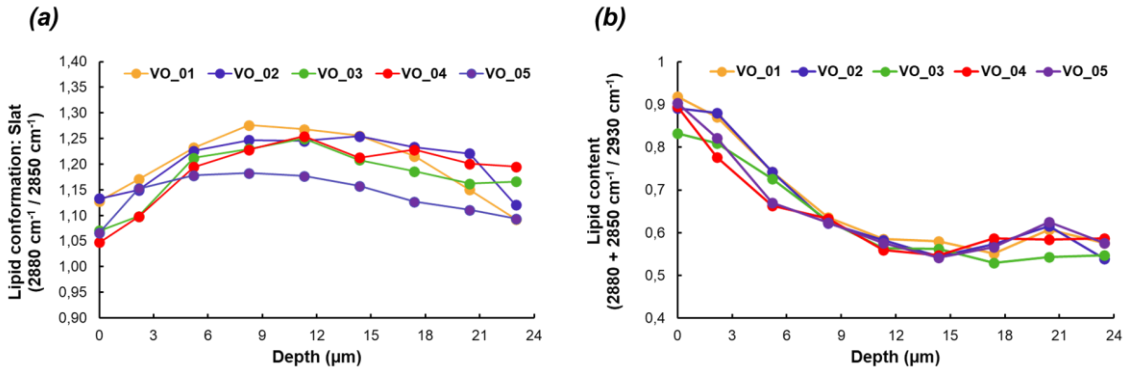

Figure 3. *(a) Calculated lipid conformation for five volonteers (VO_01 to VO_05); (b)* Calculated lipid content for five volonteers *(VO_01 to VO_05).*

Altogether, in vivo Raman spectroscopy enables detailed, reproducible, and contact-free assessment of hydration and lipid structure at a spatial resolution previously inaccessible with conventional non-invasive techniques. This molecular-level insight provides a high-value platform for research and development in dermocosmetics, particularly by fast-tracking efficacy testing through direct and quantitative markers, reducing or replacing the need for subjective evaluations or invasive procedures, and supporting the personalization of product development based on measurable skin biomarkers. By integrating Raman spectroscopy into their workflows, cosmetic R&D teams can significantly enhance formulation precision, better target consumer needs, and strengthen the scientific foundation of regulatory documentation.

4. Discussion

The results obtained through *in vivo* Raman spectroscopy not only confirm its ability to characterize key physiological and molecular parameters of the skin but also highlight its versatility for cosmetic and dermatological research. The hydration profiles observed align closely with known biophysical gradients, validating Raman's accuracy in mapping water content distribution. Compared to classical methods such as corneometry or TEWL, Raman provides depth-specific data that allows for more nuanced interpretations of how products affect the skin barrier over time. The lipid-related findings (both in conformation and concentration) provide a window into the dynamic state of the skin's extracellular matrix, which is often altered by age, environment, or pathology. These parameters are rarely accessible via traditional non-invasive methods. Raman's ability to quantify and localize these changes in vivo represents a paradigm shift in cosmetic evaluation — moving from surface-level measurements to molecular biomarkers of function and structure.

From an industrial perspective, the ability to rapidly and non-invasively evaluate: Barrier integrity, Lipid organization, Hydration retention, is a substantial advantage in the race to develop next-generation cosmetic products. These insights can guide ingredient selection, optimize formulation delivery, and support claims validation with robust scientific data. Moreover, as consumer demand shifts toward personalized skincare, Raman spectroscopy offers a scientific foundation for customizing treatments based on individual skin profiles, moving closer to the future of precision dermatology and cosmetics.

5. Conclusion

This work demonstrates the power and versatility of In Vivo Raman Analyzer for high-resolution, non-invasive skin analysis. By leveraging its molecular sensitivity and axial precision, we were able to extract meaningful data on hydration dynamics, lipid content, and lipid structure, as well as accurately determine stratum corneum thickness across multiple subjects. These measurements provide critical biomarkers for evaluating skin barrier function and cosmetic efficacy, enabling the objective monitoring of formulation performance under realistic, *in vivo* conditions. The use of a 660 nm laser at 18 mW, along with advanced optical components and spectral processing tools (LabSpec6), ensured safe, reproducible acquisition of high-quality data across all volunteers. The results highlight Raman spectroscopy's potential to transform cosmetic R&D by delivering quantifiable insights into skin physiology, while supporting personalized product development based on real-time biophysical profiling.

In the context of growing consumer expectations and regulatory scrutiny, Raman spectroscopy emerges as a cutting-edge, ethical, and scalable solution for next-generation dermocosmetic innovation.

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