Use of reflectance near-infrared spectroscopy to investigate the effects of daily moisturizer application on skin optical response and barrier function

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Abstract. A number of noninvasive techniques and instruments have emerged over the years allowing much progress toward clarifying the structure and function of human skin and studying the effects of various applied substances. All of this research has provided great insight into the interactions between skin and various products through quantitative and qualitative measurements. Such methods include near-infrared spectroscopy (NIRS), a technique which has gained popularity over the years and has often been employed to accurately determine the moisture levels and water content of skin based on its sensitivity to hydrogen bonding. NIRS has also been applied in many studies to report the efficacy of moisturizing products and assess their benefits to the skin. However, many of these studies have reported an increase in skin water content following moisturizer application while some have challenged the benefits of long-term moisturizer use, particularly on normal skin, and even suggested that it can increase the skin's susceptibility to irritants. This paper reports the results of a pilot in vivo study carried out on the skin of 20 healthy volunteers, categorized into groups depending on their skin type and frequency of moisturizer use, in order to investigate the optical response of human skin after direct short-term contact with water followed by application of a moisturizer. The measurements were obtained using a highly advanced spectrophotometer in the region of 900 to 2100 nm equipped with a customized reflectance fiber optic handheld probe. Scatter graphs of group results and second derivative spectra have shown an interesting pattern between frequent users of moisturizers and individuals who do not use moisturizers, suggesting that long-term daily moisturization may have an effect on skin barrier function. The results also raise some questions regarding the optical characteristics of different skin types, as well as the varying response between different water bands in the NIR region. Future work will focus on gaining more knowledge about these subjects and obtaining results from a larger population, as well as performing statistical analysis through regression methods in order to further improve optical skin measurements.

Keywords: near-infrared spectroscopy; water content; skin barrier function; moisturization.

1 Introduction

The study of skin hydration and skin barrier function have long been areas of great interest and extensive research in the fields of cosmetics and dermatology due to their importance as biophysical parameters of skin health.[1–3] In humans, skin features play a vital role in allowing humans to survive in a terrestrial environment by delicately regulating the amount of water loss from the skin through its outer epidermal layer known as the Stratum Corneum (SC).[1,3] The nature of this layer is complex, consisting of several layers of dead keratin-filled squamous cells called corneocytes, embedded in an intercellular lipid matrix, and acting together as an essentially selective permeable, heterogeneous, composite outer layer of the epidermis that controls the level of transcutaneous water loss and provides protection against desiccation and environmental challenges.[1,4,5] Moreover, the water content of the SC layer allows it to maintain its flexibility and facilitates the occurrence of certain enzymatic reactions which are responsible for driving the SC's exfoliating process and regulating the epidermal barrier function. The level of SC hydration or water-retaining capacity is highly dependent upon the structure and arrangement of the intercellular lipid matrix, and on natural moisturization factors (NMFs) which comprise water-soluble substances. It is also dependent on the permeation path length through the SC. In turn, barrier function is influenced by the water content of the skin which regulates hydrolytic enzyme activities involved in SC maturation and desquamation of corneocytes.

In some cases, the skin barrier function becomes impaired causing irritation and dry skin, and hence requires the application of moisturizers to treat the condition by locking water inside the SC and creating an extra barrier that prevents oils from escaping and harmful elements from entering.[6] Moisturizers are also commonly used for cosmetic purposes to improve the appearance and feel of skin. However, some studies have suggested that long-term use of certain moisturizers or overhydration can compromise the integrity of the SC layer, weaken

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skin barrier function, enhance susceptibility to irritants, and influence skin barrier recovery.[7–10] Therefore, more knowledge about the effects of moisturizing agents on the skin and its barrier function is required.

Although water is an important and vital component of human skin, prolonged skin contact with extrinsic water can impair the skin barrier function, act as a potential strong irritant, and can even lead to skin damage.[11–13] Skin contact with extrinsic water for extended periods of time can strip the skin of its natural oil and moisturizers and lead to a number of profound effects such as SC swelling, increasing SC suppleness, weakening SC corneocyte cohesion, and increasing the permeability of all substances that penetrate the skin.[14,15]

This paper reports the initial findings of a pilot in vivo experiment conducted on a small number of volunteers where the optical properties of skin were assessed after direct short-term contact with extrinsic water, and then after application of a moisturizer. The measurements were recorded using a highly advanced spectrophotometer equipped with a customized fiber optic handheld probe to obtain near-infrared (NIR) reflectance spectra of skin.

This particular technique was employed here due to its ability to directly and accurately detect water inside the skin using the intensities of overtone and combination bands of OH and HOH water bonds occurring in the NIR region, which are good indicators of the level of skin hydration and water content.[16–19] NIR spectra can also provide additional insight regarding other skin constituents including lipids and proteins, can differentiate between different types of water in the skin,[17,18] and monitor changes in skin water mobility.[2] Furthermore, this technique has the added advantage of great flexibility, allowing both in vitro and in vivo assessments to be carried out since its instrumentation can easily be equipped with fiber optic probes.

Unfortunately, a reliable instrument based on NIR spectroscopy (NIRS) designed specifically for skin hydration measurements is not yet available, although a few recent studies have looked at various ways to improve or miniaturize this technique.[20–23] Results from this experiment will be used in conjunction with our previous work[24–26] to aid the future design and development of a portable NIR spectroscopic device that would allow accurate, fast, and noninvasive in vivo measurements of skin hydration and water content.

2 Materials and Methods

Twenty healthy volunteers (5 male, 15 women; age range: 17–53 years old; mean age: 26.1 years; SD: 8.0387) were recruited and asked to complete a skin health questionnaire prior to performing any measurements. The study was approved by City University London Senate Research Ethics Committee, and all participants gave written informed consent before any personal details were collected. Volunteers with obvious skin pathology, deep suntan, or any other condition with the exception of dry legs, were excluded. For female participants, some had shaved, waxed, or used laser hair removal in the past, but this had been carried out at least a day before taking part in the study. As for male participants, hair interference was easily avoided as most did not have hair on the selected sight near the ankle and the experiment did not consider or examine the differences in skin roughness between male and female participants.

2.1 Experimental Design

The participants were categorized into four groups depending on their skin type and frequency of moisturizer use: (Group 1) comprised those who apply moisturizer daily on the test area and have normal skin; (Group 2) also included those with normal skin, but that do not use moisturizers on the test area; (Group 3) consisted of individuals who moisturize daily or on most days, but have dry skin (most with a history of eczema/dermatitis, asthma, or allergies); and last, (Group 4) included individuals with random patterns of moisturizer application and varying skin types. Each group consisted of five participants and was a mixture of skin colors, classified in the questionnaire using categories that reflect the Fitzpatrick skin color scale.

For skin moisture measurements, it is common to carry out tests on the lower leg area as many studies have done in the past.[17,18,27–30] In this case, readings were obtained by placing a customized reflectance probe slightly above the ankle on the internal lower left leg of each volunteer, then taking three spectrophotometric scans for each measurement for averaging purposes. Spectra were recorded from participants at three different intervals. Three averaged spectra were obtained from each volunteer and used for further analysis. The first set of recorded spectra, which were later averaged into a single spectrum for each volunteer, was considered as the control measurement, where the skin of the individual was at its natural state (without undergoing a wash-out period or application of tape stripping, moisturizer, or wet patch). Once this first reading was obtained, a wet patch previously immersed in water was immediately placed on the same site and left on the skin for 10 min. Then the patch was removed and the skin surface was gently tapped with a paper towel to remove any residual water before immediately replacing the probe on the same site to obtain the second set of measurements. After taking this measurement, the skin was left for an extra minute to dry in order to ensure that it was no longer wet from the patch. Participants were asked to take a roughly quarter-sized amount of a commercial moisturizing agent with their finger and rub this on the test site without leaving any residue. After 30 min, a third set of spectral scans were recorded. All participants used the same moisturizer whose list of ingredients stated on the packaging were as follows: Aqua, Cyclopentasiloxane, Sorbitol, Caprylic/Capric Triglyceride, Stearic Acid, Helianthus Annuus Hybrid Oil, Isohexadecane, Glycol Stearate, Dimethicone, Collagen Amino Acids, Isomerized Linoleic Acid, Lactic Acid, Potassium Lactate, Sodium PCA, Urea, Serica Powder, Hydrolyzed Silk, Dimethiconol, Stearamide AMP, Glyceryl Stearate, Cetyl Alcohol, Polysorbate 80, Sorbitan Oleate, Triethanolamine, Carbomer, Xanthan Gum, Sodium Acrylate/Sodium Acryloyldimethyl Taurate Copolymer, Parfum, Benzoic Acid, Imidazolidinyl Urea, Methylparaben, Phenoxyethanol, Propylparaben, Sodium Benzoate, Alpha-Isomethyl Ionone, Benzyl Alcohol, Citronellol, Coumarin, Hydroxyisohexyl-3-Cyclohexene Carboxaldehyde, Limonene, Linalool, and CI 77891.

2.2 Instrumentation

NIR skin spectra were collected using the Lambda 1050 dual beam UV/Vis/NIR spectrophotometer (PerkinElmer Corp, Waltham, Massachusetts) at increments of 2 nm in the spectral region of 900 to 2100 nm, with an InGaAs detector operating throughout the entire region. Light was provided by a tungsten

lamp and a gain setting of three on the InGaAs detector was also added to measure the energy in the single-mode beam and give better quality spectra. The scanning period for each interval was set at 0.2 s; this value seemed to give a reasonable balance between sensitivity and scan time. Therefore, each scan lasted 2 min, and three cycles were performed so that each averaged spectrum was taken over a period of 6 min for each test.

Attenuator settings were kept constant at 1% for the reference beam to improve the noise levels at high absorbance and were maintained at 100% attenuation for the sample beam. Slit size controls for the InGaAs detector, which allows one to adjust the amount of light entering, was set on "servo mode" so that the system could oversee the reference beam energy and select the slit size accordingly.

Initial baseline corrections were performed to eliminate the irrelevant bands and background noise which especially evident in highly absorbing media such as skin. These corrections were carried out at 100% T/0A baseline to correct the maximum sample and reference beams, and at 0% T/blocked beam to regulate the beams at 0% for the sample and 100% for the reference.

In order to acquire reflectance measurements, it was necessary to replace the sample holder compartment with a universal reflectance accessory that permitted the attachment of a fiber optic probe (Ocean optics, Duiven, The Netherlands). This probe consists of a bundle of seven optical fibers in a stainless steel ferrule with six of them arranged around one light detecting fiber and acting as illumination fibers. Depending on the connection of the probe to the spectrophotometer, it was possible to use the single fiber as the source of radiation and the other six fibers as detectors. The fiber core diameter was 600 μm, allowing a fairly reasonable area of skin to be sampled.

It was essential to introduce a small gap between the probe and the test site to prevent the blockage of light leaving and entering the probe, since occlusion can lead to a build up of water and increase hydration, thereby falsely raising skin water content measurements. Therefore, the reflectance probe was modified by enclosing its tip with a Perspex tube layer that was longer than the end of the probe by 1.5 mm. This coating ensured that the desired separation distance was maintained throughout all tests. In addition, this particular coating was overlaid with another white coating to eliminate interference from ambient light while scanning. Figure 1 shows this probe placed on a volunteer with the separation distance indicated.

All spectra obtained were transferred from the standard PerkinElmer UV Winlab software (Perkin ElmerCorp, Massachusetts, USA) to the UVWinlab Data Processor and Viewer software (Perkin ElmerCorp, Massachusetts, USA) in order to perform pretreatment processes such as averaging and smoothing as well as standard normal variate (SNV) scatter corrections. For qualitative analysis of data, principal component analysis (PCA) was performed with Multibase 2014 Excel plugin (Numerical Dynamics).

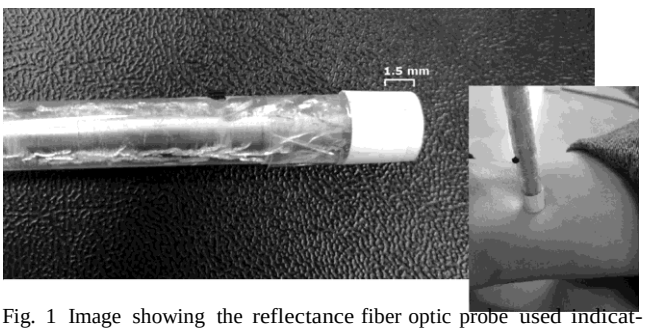

Fig. 1 Image showing the reflectance fiber optic probe used indicating the separation distance introduced and as applied on a participant.

3 Results

Skin reflectance spectra obtained with the fiber optic probe were transformed into absorbance using the equation Log $1/R$, prior to undergoing smoothing, averaging, and scatter correction using the SNV method. Smoothing was applied using the block average method with a window of 50 data points in order to maximize the noise reduction. For the three set of spectral measurements taken from each participant (control, after application of a wet patch, and then 30 min after treatment with a moisturizer), the average was calculated again for each set to give the mean of the mean of all data. This graph is shown in Fig. 2. All the spectra in this graph clearly display the dominant OH stretching overtone and combination bands of water at 1450 nm and between 1910 and 1920 nm, as well as a shoulder around 2000 nm indicative of bound water, and a weaker band at 1760 to 1780 nm arising from alkyl CH groups in skin lipids and protein. However, other bands belonging to NH groups are less visible and seemed to have merged with those belonging to water.

Comparing the three resulting spectra averaged from all participants, the overtone band around 1450 nm is greater for the first measurement (control), recorded before the application of the wet patch or moisturizer, which indicates higher water content. However, at the combination band of OH and HOH bending in the region of 1900 to 1920 nm, all three spectra are nearly equal, although the average spectrum obtained after applying the wet patch was slightly dominant in this particular region.

It appears that spectra showing a smaller intensity at the water absorption bands had more prominent features at peaks belonging to NH and CH groups in correlation to spectra where water absorption was dominant, but the baseline of the spectra was not significantly different among the three recorded measurements.

Before looking into the results from the wet patch and moisturizer tests, PCA was performed for the control measurement which was taken from volunteers at their normal state. The analysis was performed using the second derivative spectra with the four groups of participants as the different variables.

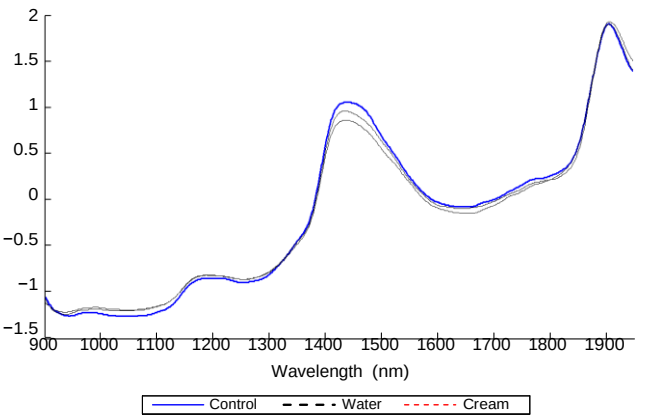

Fig. 2 Mean of the mean absorption graph of all volunteers before, and after application of a wet patch followed by a moisturizer.

Derivative spectra were calculated on the UVWinlab software using the Savitzky–Golay method. PCA was performed to reduce the complexity of the dataset and to determine whether a pattern existed among individuals with consistent, nonconsistent, and no moisturizing routines. In order to focus the analysis on the water-related bands, only spectral values between 1300 to 1600 and 1750 to 2050 nm were included. The resulting scatter plot of the samples' scores is shown in Fig. 3. The minimum of the 1450 nm band contributed mostly to the loadings of the first principal component (PC1), whereas the minimum of the 1900 nm water band dominated that of the second principal component (PC2). Overall, these two principal components accounted for 40% and 25% of the total variation, respectively. The scores' plot in Fig. 3 clearly distinguishes among groups with different moisturizing patterns, with those from Group 1 showing the least variation. Moreover, the plot shows a marked increase in separation as the frequency of moisturizer applications decreases, regardless of skin type.

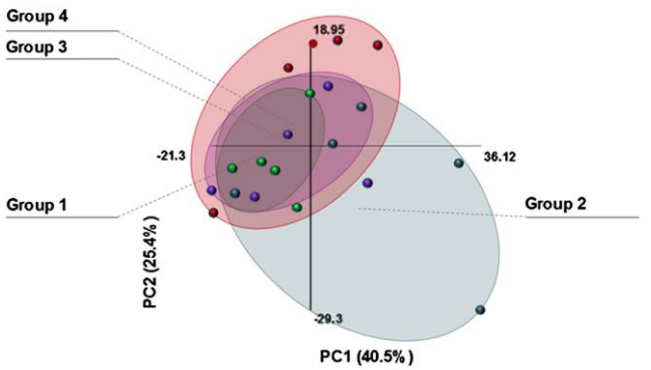

Fig. 3 Scores plot of PCA analysis performed on the control measurements of individuals with varied moisturizing patterns.

Then, peak values at the 1900-nm water band were analyzed using the post-treated absorption spectra from all participants. This particular band was chosen because previous studies[2,31] have reported that the 1900-nm water band is a more suitable indicator of the state of water molecules in the SC than the 1450 nm band, which is often influenced by adjacent protein bands. Therefore, peak values from the band near 1900 nm

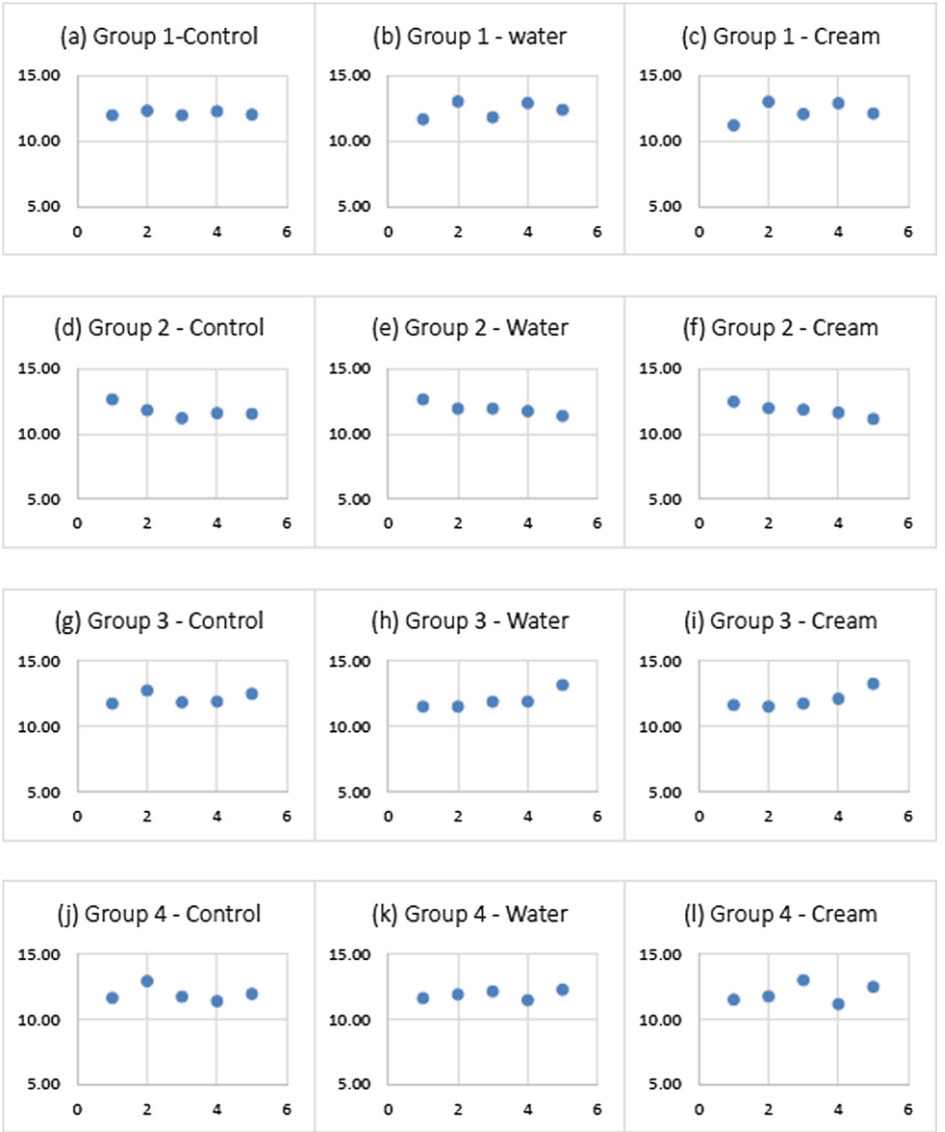

Fig. 4 Scatter graphs of group peak values at 1900 nm for each test performed.

were plotted on a scatter graph (Fig. 4) for each group and each test performed (control, water, and cream). In accordance with PCA results, the control measurement for Group 1, which consisted of individuals with normal skin and who moisturized daily, showed the least variation between individuals compared to the results from other tests and groups. However, this was different for the other two tests performed, although both graphs [Figs. 4(b) and 4(c)] yielded similar patterns. This same pattern consistency in the water and cream tests was also evident for individuals in Group 3, but there was a difference in their initial control measurement. Overall, the results of individuals with normal skin and that do not moisturize (Group 2), varied the least between all tests conducted and generally yielded a similar pattern when observed on scatter graphs. Results from Group 4 individuals were random, with no obvious trend detected, however, this was expected since this group consisted of individuals with different skin types and moisturizing routines. For this reason, further analysis from this point was restricted to Groups 1–3 to allow the investigations of groups that could be easily categorized in terms of skin type and moisturizing routine.

In addition, it is known that NIRS is capable of providing more in-depth recordings of skin because its light can penetrate deeper into the skin. It has been reported[32] to travel an average path length of 1.5 to 2.8 mm in the region between 1400 and 1900 nm, reaching the topmost part of the dermis. However, the beam path length in media is highly influenced by wavelength since optical properties such as scattering and absorption coefficients and anisotropy parameters are the functions of wavelength. Previous studies[33] have concluded that the water bands at 1450 and 1900 nm visible in skin spectra have different absorption coefficients, and, therefore, have a different measurement depth. The water band around 1900 nm was shown to have a higher absorption coefficient, resulting in a shorter measurement in comparison to the band near 1450 nm since photons lose their energy more rapidly in the spectra range of strong water absorption. To investigate this point, the second derivative of spectra shown in Fig. 2 was used again instead of the absorption curves to minimize the errors from baseline fluctuations that occur in absorbance measurements as a result of changes in optical parameters, such as the scattering coefficient. These are shown in Fig. 5. Looking at this figure, there was no apparent shift in water absorption bands or any other bands from the three recordings and for each after contact with water or after moisturizer application. However, the peak intensity of local minimum at 1463 nm, which corresponds to the overtone water absorption band at 1450 nm, was highest for the control measurement, indicating increased water contents. The peak intensity of a local minimum at 1901 nm, related to the water absorption at 1900 nm, was highest in the spectrum recorded after treatment with cream. However, the 1450 nm band is influenced by two bands around 1400 and 1500 nm resulting from the combination of CH-stretching and CH-bonding modes and the first overtone of the NH_2-stretching mode, making it less suitable for accurate water determination. Peak height differences at this minimum were also smaller than those at 1463 nm.

The same derivative calculations were then repeated, this time separating the resulting spectra of Groups 1–3 at each setting to investigate their individual response. These results are shown in Figs. 6–8. Again, the intensity of the local minimum at 1463 and 1901 nm did not reflect each other, probably because of the influence of proteins at 1450 nm and differences in measurement depths. It can be noted that these bands almost have an opposing behavior. Second derivative spectra from Group 2 consistently showed the highest intensities for local minima at 1463 nm in comparison to Groups 1 and 3, and the lowest intensity peaks for local minimum at 1901 nm. On the other hand, the derivative spectra of Group 1 are nearly the opposite of this, mostly having smaller intensities at

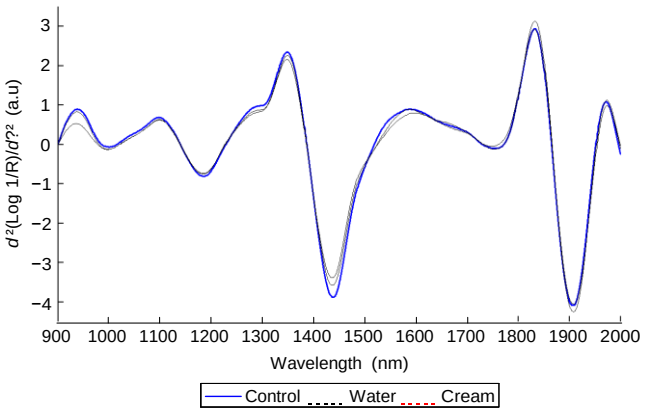

Fig. 5 Second derivative spectra of mean of the mean absorption graph (Fig. 2) of all volunteers before, and after application of a wet patch followed by moisturizer use.

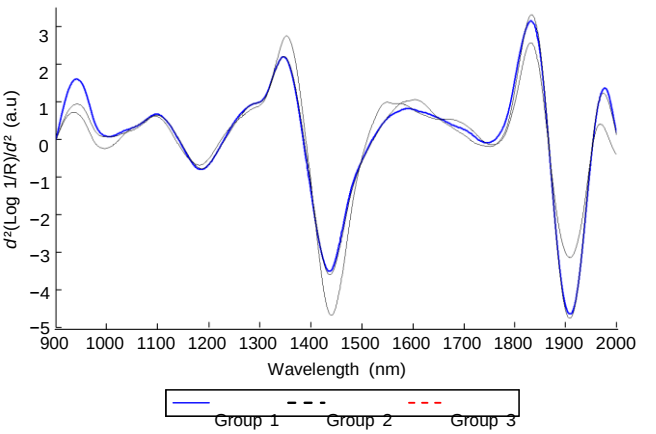

Fig. 6 Second derivative spectra at control setting (taken before applying wet patch or moisturizer) for Groups 1–3.

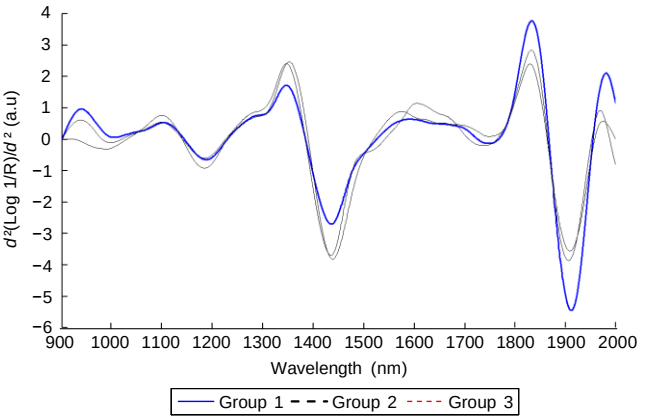

Fig. 7 Second derivative spectra after applying a wet patch for 10 min. Response for Groups 1–3.

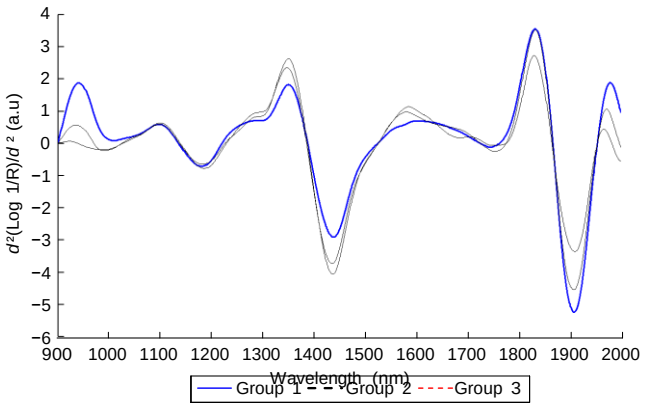

Fig. 8 Second derivative spectra after treatment with a cream. Response for Groups 1–3.

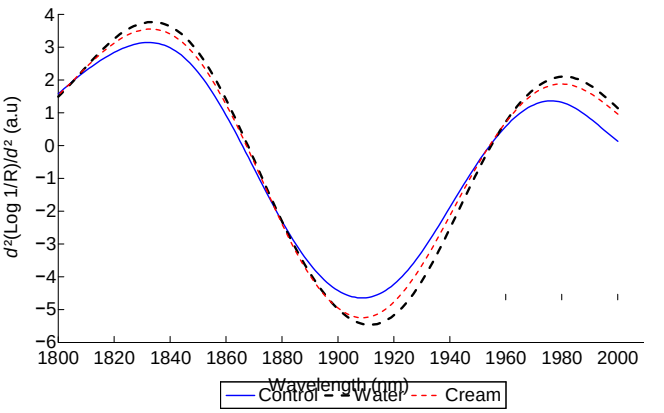

Fig. 9 Second derivative spectra of Group 1 for the three measurements recorded.

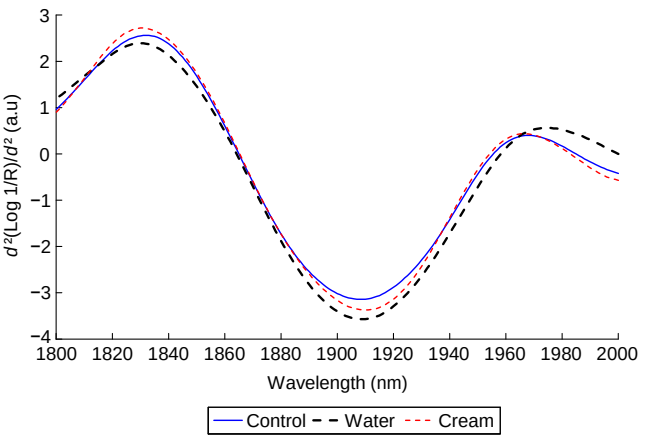

Fig. 10 Second derivative spectra of Group 2 for the three measurements recorded.

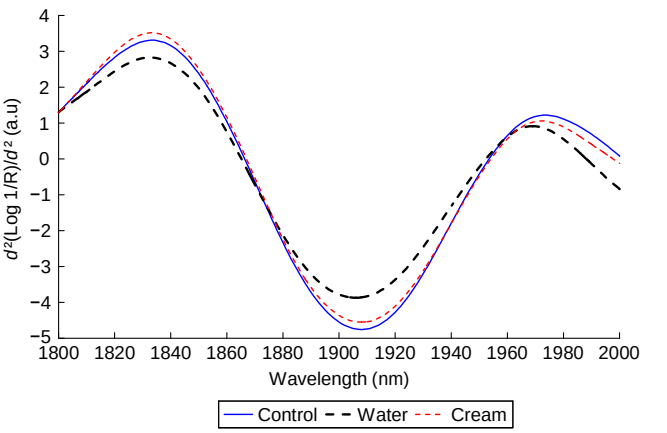

Fig. 11 Second derivative spectra of Group 3 for the three measurements recorded.

1463 nm and the largest at 1901 nm, except at the control setting where the intensity peak at 1901 nm for Group 3 very slightly exceeded that of Group 1 although they are nearly identical. Overall, spectra obtained after application of a moisturizer showed the least amount of variation between groups, although showing the same pattern as explained above. Therefore, since previous studies[34] have shown that the light beam would have traveled deeper into the skin at the shorter wavelength, i.e., around 1463 nm, it can be assumed that individuals from Group 1 who normally moisturize their skin on a daily basis had a higher concentration of water at the more superficial parts of the skin, whereas for participants in Group 2, the opposite would be true and water was able to travel deeper into the skin. As previously reported in the literature,[2,18,35] NIR skin spectra can also be used to investigate changes in skin water mobility by monitoring a possible peak shift at around 1900 nm, which is dependent on the skin condition and environment. It was also reported[2,35] that an increase in the skin water content causes an increase in water mobility marked by a peak shift toward shorter wavelengths at the 1900 nm water band. Therefore, the procedure outlined by Egawa[2] was applied here to analyze the water mobility in the SC for Groups 1–3, and to examine the differences between each group. In this case, the second derivative spectra from each group were analyzed separately for the three tests performed and are shown in Figs. 9–11. As illustrated by these spectra, Group 1 expressed no changes in water mobility between the control reading and those taken after moisturizer application, but a small peak shift toward a longer wavelength (1909 nm to 1911 nm) did occur after the water test. As for Group 2, minor peak shifts towards longer wavelengths were observed at the 1900 nm band in spectra recorded after the wet patch test and after moisturizer application. This indicates a slight decrease in water mobility. In contrast, moisturizer use failed to cause a peak shift in individuals belonging to Group 3; however, a small shift from 1908 to 1906 nm did occur as a result of the water test, this time indicating a slight increase in water mobility.

Finally, PCA was performed again, this time on the mean second derivative spectra from Groups 1–3, and for all three tests (control, water, and cream). Again, only spectral values measured between 1300 to 1600 and 1750 to 2050 nm were entered into the analysis to narrow the model to the water-related regions of skin spectra. Figure 12 shows the resulting scatter plot of sample scores. Here, the first and second principal components, PC1 and PC2, accounted for 59% and 19% of the overall deviation, respectively, and have clearly separated the three groups regardless of the tests performed. However, the closest relationship remains between the control measurements of

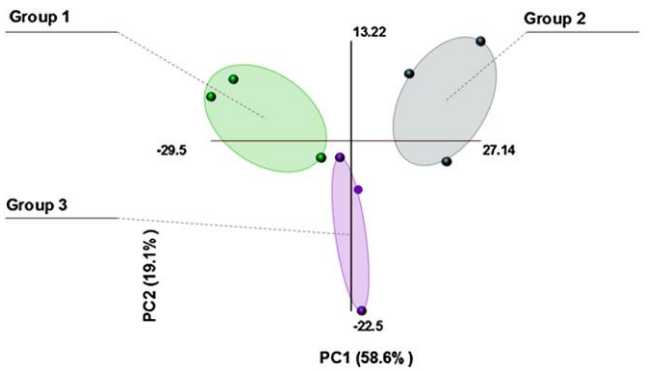

Fig. 12 Scores plot of PCA analysis performed on the averaged second derivative spectra from Groups 1 to 3 for all tests carried out on volunteers.

Groups 1 and 3, thus highlighting the obvious influence of daily or frequent use of moisturizers.

4 Discussions and Conclusions

A state-of-the-art spectrophotometer equipped with a customized fiber optic reflectance probe was employed to perform an in vivo experiment investigating the effects of short-term direct water contact with skin and application of a common moisturizer on a small group of participants divided into categories depending on their type of skin and frequency of typical moisturizer use. Looking at the mean spectra of all participants for the three tests, it was evident that the resulting spectrum from recordings taken prior to contact with water or treatment with a moisturizing agent showed higher peak values around the OH overtone band of water near 1450 nm, which is probably expected since the majority of volunteers were frequent users of moisturizers. However, this was not the same for the OH combination band of water around 1900 nm, where peak values from all tests were quite similar in that region.

Second derivative spectra have shown an interesting pattern between frequent users of moisturizers and individuals who do not moisturize. Participants who do not use moisturizers consistently had the lowest intensity at 1901 nm, but the highest at 1463 nm, and since previous studies[34] have shown that measurement depths of NIR light largely depend on water absorption and that higher water absorption leads to shorter light path lengths, the beam would have traveled deeper into the skin at the shorter wavelength. Based on this, it may be possible to suggest that individuals who moisturized daily (Group 1) had a higher concentration of water at the more superficial parts of the skin, whereas for the other group (Group 2), the opposite would be true and water was able to travel deeper into the skin. However, results from a larger population are required to give further insight into this hypothesis.

PCA results were able to yield valuable clusters of the data. The first of those (Fig. 3) showed that randomization and separation between individuals increased with decreased moisturizer application with little consideration for skin type. A second PCA (Fig. 12) scores plot, made up of the averaged second derivative spectra of Groups 1–3 performed including all tests that were carried out, was reasonably able to distinguish among the three groups, but showed moisturization patterns to be an apparent influential factor since the control measurement from Groups 1 and 3 had the closest overall relationship.

In conclusion, it seems many questions remain regarding the optical properties of different skin types and the influence of frequent moisturizer use, as well as the relationship between different water absorption bands in the NIR in relation to moisturizer use. Future work will focus on acquiring data from more subjects and employing statistical regression methods for more in-depth analysis of these results in order to improve optical and spectroscopic skin measurements, and hopefully support the design and development of a portable and/or miniaturized optical device that could provide reliable, accurate, and fast skin hydration readings in vivo to eliminate the use of current bench-top instruments with the same performance standards used today.

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