Depth-profiling of skin by near infrared spectroscopy

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Introduction

The outermost layer of skin, the stratum corneum (SC), is only about 20–50 micrometers thick, yet serves a critical role in regulating the exchange of water between the body and its environment. The SC consists of layers of keratinized cells surrounded by lipid bilayers. It is these lipid bilayers which are thought to be primarily responsible for the water-holding function of the SC and its water permeability.[1]

Because the SC undergoes a continuous process of cellular turnover, there is great variability in composition between the deeper and surface layers. At the base of the SC, living cells extrude lipids and cornify into flattened, anucleate cells which are shed as they reach the surface. The composition of the SC lipids also undergoes change as enzymatic and hydrolysis reactions may occur. Water content, based on *in vitro* studies, is generally about 10–30% within the SC under normal humidities, with higher concentrations found in the deeper tissue.[2] Below the SC there is an abrupt increase in water content to about 70%.[3]

Water is associated with both major components of the SC, the lipid bilayers and the keratin. Differential scanning calorimetry on isolated SC sheets indicates that 33% of the SC consists of bound (unfreezable) water but that this level fluctuates with lipid content.[1] Keratin also provides a site for water. Infrared (IR) and nuclear magnetic resonance (NMR) *in vitro* studies indicate that hydration occurs on the polar sites of keratin, with secondary hydration occuring as the relative humidity increases.[2,4]

Previous work in this laboratory showed that near infrared (NIR) reflectance could distinguish four types of water in skin.[5] These are (i) water associated with the lipid phase within the SC at 1875 nm, (ii) bulk water below the SC at 1888 nm, (iii) secondary water of hydration on SC keratin at 1905 nm and (iv) primary water of hydration on SC keratin at 1923 nm. These assignments were based on behavior under varying RH conditions, *in vitro* studies on pigskin and studies on hair keratin and model systems.

In the present study, we have attempted to use near infrared reflectance to map the variability in water content across the SC *in vivo*. Two approaches were employed: one was to vary the distance between the sample (skin) and the detector and the other approach was to vary the aperture at the detector port.[6] Both approaches are based on the idea that radiation which is back-scattered from deeper tissue on average reaches the surface further from the point of incidence than radiation back-scattered from shallower tissue. In addition, the stratified layers of the stratum corneum are likely to cause multiple scattering, which will also result in radiation from the deeper layers re-emerging further from the source.

Experimental

Depth profiling was performed on the inner forearm of a caucasian female using an LT Industries Quantum 1200 grating spectrometer in the diffuse reflectance mode. Thirty scans per spectrum were collected and 4–8 spectra were averaged under each condition to minimize variation due to slight changes in arm placement over the detector port. No window was used in the detector port in order to avoid occlusion of the skin, which could affect water content. A lycra mousepad was used as a homogenous sample to study wavelength effects in both depth profiling experiments.

For the distance experiment, anodized steel cylinders (i.d. 29 mm) were inserted into the detector port to provide distances of 0, 2, 4, 6, 8, 12, and 16 mm. For the aperture experiment, rings of black emery paper (SiO_2) were placed over the detector port to provide openings of 800, 660, 450, 240, 130 and 60 mm^2. Background spectra of a ceramic tile were acquired under the same conditions as the corresponding sample in order to avoid unmatched pathlengths or areas.

Results

Skin

The assignments for the 2nd derivative near infrared bands are given in Table 1.[5] The intensities used in all Figures are determined from 2nd derivative spectra.

The band at 1888 nm should act as a measure of penetration depth since it lies below the SC. Deeper penetration should thus result in a stronger signal at this wavelength. Figure 1 shows the intensity at 1888 nm plotted against distance from the detector at three different humidities. In each case, the decrease in intensity with increasing distance is initially strong, then levels off. Figure 2 shows a similar plot for aperture area (at one humidity). Here, the decrease in intensity appears to be linear with a decrease in area, indicating that the 1888 nm band is probably a good measure of penetration depth. From the range of intensities for the 1888 nm band in Figures 1 and 2, it is apparent that the area experiment (100–1400) covers a much greater range of depths than the distance experiment (600–1000).

Figure 3 shows the progressive decrease in SC water concentration between the deeper and shallower tissue in the area experiment. The distance experiment (not shown) indicates the same trend. The lipid-associated water (1875 nm) appears to drop off more rapidly than the protein-water (1905 and 1923 nm). Note that the primary and secondary water on protein maintain a constant relative concentration throughout this interval.

In Figure 4, the intensities of the bands for lipid-associated water (1875 nm) and lipid alkyl chains (1722 nm) are plotted against the intensity at 1888 nm (a measure of penetration depth).

Table 1. Assignments of NIR bands in skin.

1875 nm	water associated with lipid in SC
1888 nm	bulk water below the SC
1905 nm	secondary water of hydration on protein
1923 nm	primary water of hydration on protein
1722 nm	primarily alkyl chains of lipids
2168 nm	protein

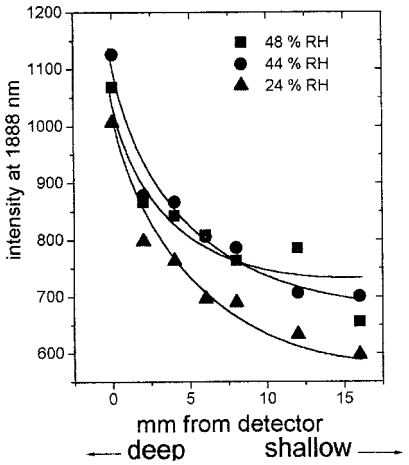

Figure 1. Second derivative intensity at 1888 nm (water below the SC) versus distance between inner arm and detector port.

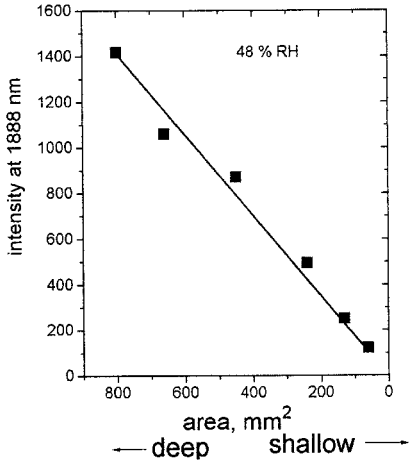

Figure 2. Second derivative intensity at 1888 nm (water below the SC) versus aperture area.

In both the area and distance experiments (the latter not shown) the water band intensity at 1875 nm drops off more rapidly towards the skin surface than does the lipid band intensity, indicating that the degree of hydration of the lipid phase decreases towards the outer surface of the SC, as expected.

For protein hydration, the result is similar (Figure 5). In the area experiment, the secondary water on protein (1905 nm) decreases more rapidly towards the SC surface than does the protein (2168 nm). The distance experiment (not shown) shows the same trend.

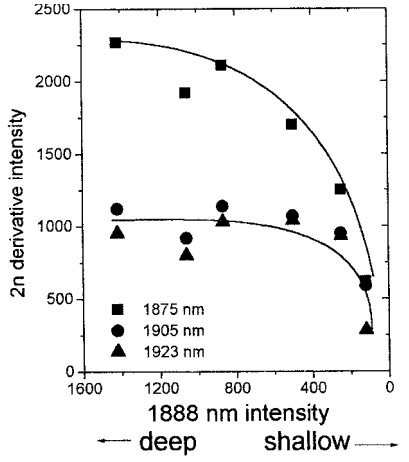

Figure 3. Second derivative intensities of SC water bands at 1875, 1905 and 1923 nm versus intensity at 1888 nm (48% RH).

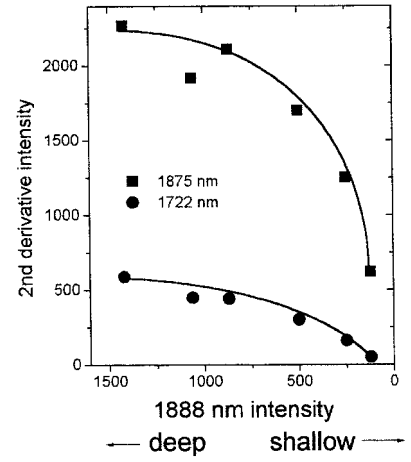

Figure 4. Second derivative intensities at 1875 (water associated with lipid) and 1722 nm (lipid) versus intensity at 1888 nm (48% RH).

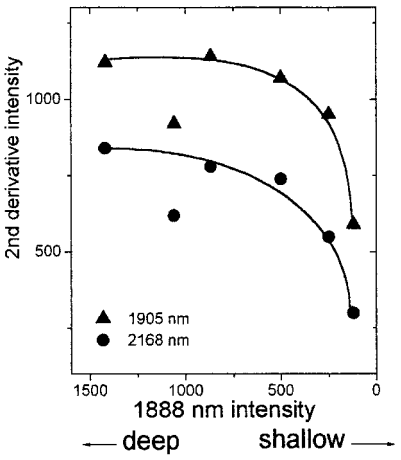

Figure 5. Second derivative intensities at 1905 (secondary water on hydration on protein) and 2168 nm (protein) versus intensity at 1888 nm (48% RH).

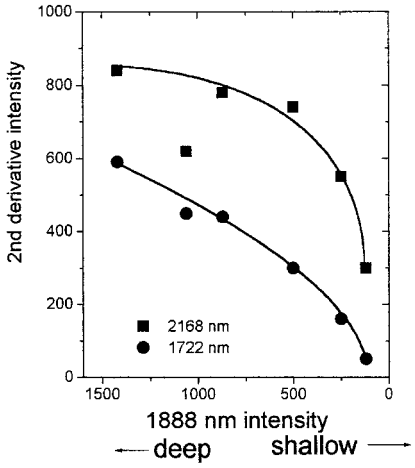

Figure 6. Second derivative intensities at 2168 (protein) and 1722 nm (lipid) versus intensity at 1888 nm (48% RH).

Figure 6 shows the intensities of the protein (2168 nm) and lipid (1722 nm) intensities against the 1888 nm intensity. Here, the protein appears to decrease more rapidly than the lipid, suggesting that there is more lipid per protein unit in the shallower SC. This result is consistent with measurements from tape-stripping experiments showing increased fatty acids in the outer SC.[7]

Wavelength dependence

The lycra mousepad was subjected to both the area and distance experiments to look for wavelength dependency effects. Figures 7 and 8 show the results for the area and distance experiments. In both, the spectra are scaled so that the 1662 nm band of lycra appears as the same intensity for each spectrum. As the distance increases or area decreases, the longer wavelength bands fall off in intensity more rapidly than the shorter wavelengths. Second derivative intensity ratios (not given) show this more clearly.

The result of this wavelength dependence is that if no change in sample composition occurs, longer wavelength bands will appear to decrease more sharply than shorter wavelength bands as the average depth of penetration becomes shallower. In the skin experiment, comparisons between wavelengths sometimes result in the longer wavelength band showing a sharper decrease at shallower depths (2168 and 1722 nm, 1875 and 1722 nm) but at other times the opposite (1875 and 1905 nm, 1905 and 2168 nm). Thus, the skin results may be influenced by wavelength effects but are not dominated by them.

Summary

Depth-profiling of the stratum corneum can be accomplished *in vivo* by either varying the distance between detector and sample, or varying the aperture at the detector. The latter approach appears to have a linear relationship between area and depth of penetration, while the first approach gives a steeper drop in penetration as the distance increases initially, which levels off at longer distances. With both methods, longer wavelengths change more rapidly than shorter ones; thus care is necessary in interpreting results.

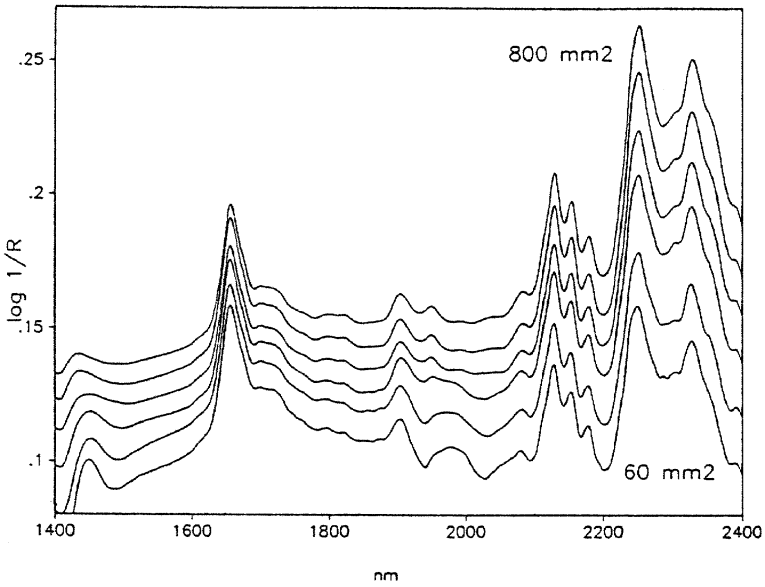

Figure 7. Log 1/*R* spectra of lycra mousepad at apertures of 800, 660, 450, 240, 130 and 60 mm^2 (top to bottom).

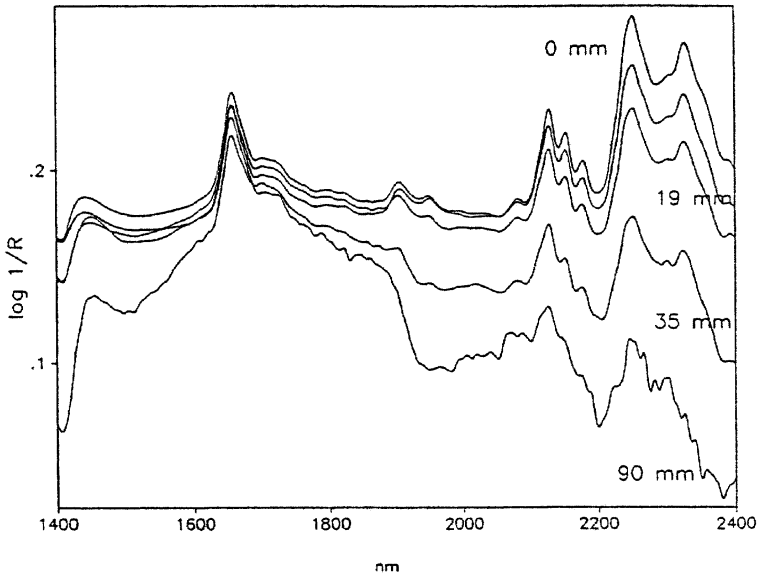

Figure 8. Log 1/*R* spectra of lycra mousepad at distances from the detector port of 0, 12, 19, 35 and 90 mm (top to bottom).

Within the SC it appears that all types of water decrease towards the surface, in accordance with *in vitro* studies reported in the literature. In addition, it appears that both the lipid and protein phases are less hydrated towards the surface (at 48% RH) while the lipid content increases relative to the protein content.

References

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