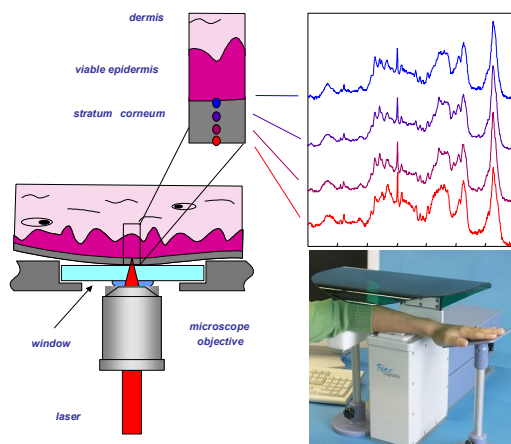


## In vivo assessment of NMF content of skin at various body sites

### Introduction

Natural moisturizing factor (NMF) is believed to be of primary importance for maintaining optimal hydration of the stratum corneum (SC). It is likely that removal of NMF may result in dry skin. For detailed studies of the effects of skin care products on skin NMF and skin hydration, noninvasive *in vivo* microscopic methods are highly desirable. Such a method is now available from River Diagnostics.

*In vivo* Confocal Raman Spectroscopy is a novel method using vibrational spectroscopy based on light scattering. River Diagnostics has recently introduced the model 3510 Skin Analyzer, a confocal Raman spectrometer optimized to directly measure living human skin. The instrument's high spatial resolution and high signal throughput enable the study of variations in the molecular composition of the skin, especially the SC, as a function of depth. In the present work the technique was applied to measure NMF content at various body sites.



**Figure 1.** *In vivo* Raman spectroscopy with the Model 3510 Skin Composition Analyzer. Near infrared laser light of low intensity is focused on the skin. The Raman spectrum of the scattered light is analyzed, revealing the local molecular composition. Molecular concentration profiles are measured by varying the position of the laser focus through the depth of the SC.

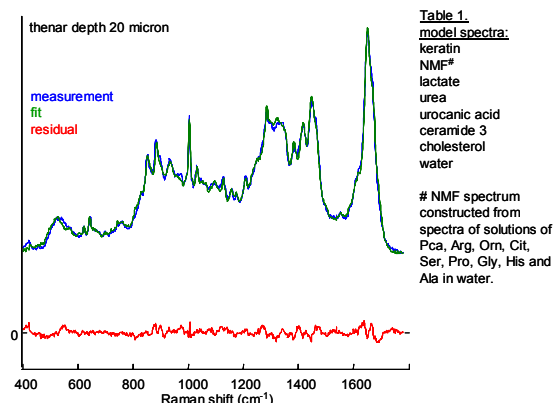
### Experimental

NMF profiles were measured on the thenar, volar forearm, volar upper arm, tip of the nose and cheek of two adult male subjects. Average NMF content over part of the SC was compared between body sites.



**Figure 2.** Raman spectroscopy offers the possibility to non-invasively look inside the skin.

Quantification of skin NMF was done by Classical Least Squares fitting of the *in vivo* Raman spectra, using a model set of *in vitro* spectra of skin components (see figure 3 and table 1). The NMF content was defined as the ratio between the fit coefficients of NMF and keratin.

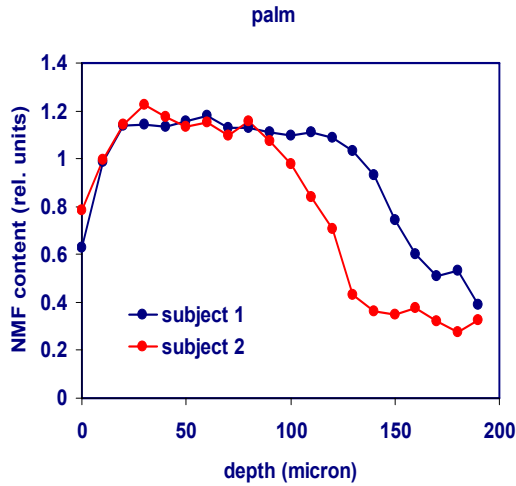


**Figure 3.** Spectrum of the thenar at a depth of 20  $\mu\text{m}$ , the result of the fit and the residual spectrum (measurement – fit).

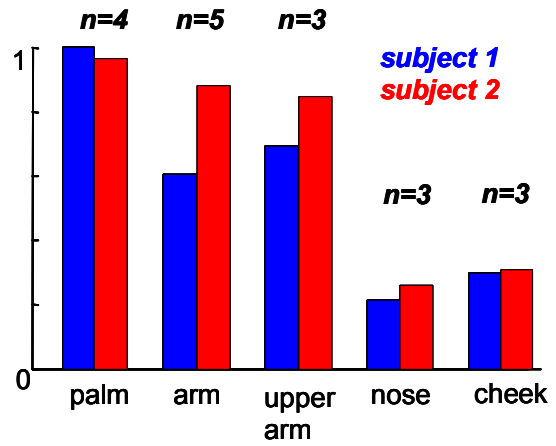
**Results**

Figure 4 shows the concentration profiles of NMF in the palm (thenar) of the two subjects. Since NMF is formed by enzyme action in the bottom-most cellular layers of the SC, NMF composition profiles are an indication of the thickness of the SC. Comparison of the two profiles in Figure 4 provides a measure of the difference in thickness of the SC between the two subjects.

Figure 5 enables a direct comparison between subjects and body sites. Shown is the NMF content averaged over a selected part of the SC (refer to the figure for detail). Remarkably different NMF content is found at different body sites, with much lower NMF content in facial skin.



**Figure 4.** NMF profiles of the thenar. Ratio of spectral contributions due to NMF and keratin, versus depth.



**Figure 5.** Average NMF content at different body sites. NMF/keratin ratio was averaged from 10-80 μm for the thenar and 0-8 μm for other sites. The number of profiles averaged per subject is indicated above the bars.

**Conclusion**

Large differences were observed between NMF concentrations at different body sites with highest NMF content on the thenar. Surprisingly, the NMF content on the nose and cheek is much lower than on hands and arms.

In vivo confocal Raman spectroscopy is a powerful, noninvasive method to obtain detailed information about molecular composition and distribution of the human skin. Because the technique is fast and non-destructive, changes and effects of treatment of the skin can be monitored over time.

**Reference**

[1] Caspers PJ, Lucassen GW, Carter EA, Bruining HA, and Puppels GJ, J. Invest. Dermatol., 116 (3), 434-442, 2001.

 <i>Instruments for Breakthrough Skin Research</i>	 <i>Partner in Product Development</i>
 <i>Contract Research</i>	 <i>Advancing Knowledge in Skin Science</i>