

## The Use of Confocal Raman Spectroscopy for the *in vivo* Measurement of NMF Concentrations in Skin

The skin's outermost layer, the stratum corneum, is the main protective barrier against water loss, microorganisms, and toxic agents. A detailed knowledge of the concentration and distribution of water and free amino acids in the stratum corneum is important for a better understanding of the properties of this layer.



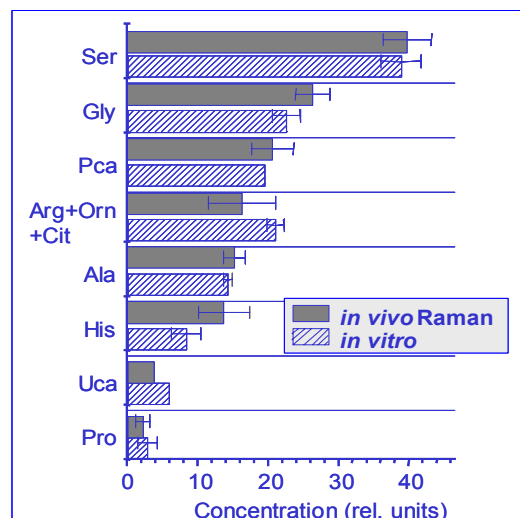
**Figure 1.** The River Diagnostics model 3510 depth-scanning skin analysis system can non-invasively analyze molecular concentration profiles in the skin.

Water greatly affects physical properties of the stratum corneum such as permeability and flexibility, and is also thought to regulate the activity of specific hydrolytic enzymes that are important for normal desquamation of corneocytes at the skin surface.<sup>1</sup> Hydration of the stratum corneum, which is exposed to the relatively dry external environment, is maintained by natural moisturizing factor (NMF). NMF is a highly hygroscopic and water-soluble mixture of amino acids and their derivatives and specific salts. NMF resides exclusively in the stratum corneum since it is produced in the lower part of the stratum corneum from an enzymatic degradation of the protein fillagrin.

<sup>1</sup> A.V. Rawlings, I.R. Scott, C.R. Harding and P.A. Bowser, *Journal of Investigative Dermatology*, 103 731-741 (1994).

Confocal Raman microspectroscopy, as implemented in the River Diagnostics model 3510 depth-scanning skin analysis system (Figure 1), can non-invasively measure molecular concentrations *in vivo* revealing gradients in the outermost skin layer. The depth resolution is better than 5  $\mu\text{m}$ .<sup>2</sup>

The Raman spectra are automatically analyzed by the RiverIcon software. Each spectrum is described as a linear combination of carefully measured spectra of pure skin components stored in the RiverIcon software. The validity of this approach has been demonstrated by comparison to classical *in vitro* methods (Figure 2).<sup>3</sup>

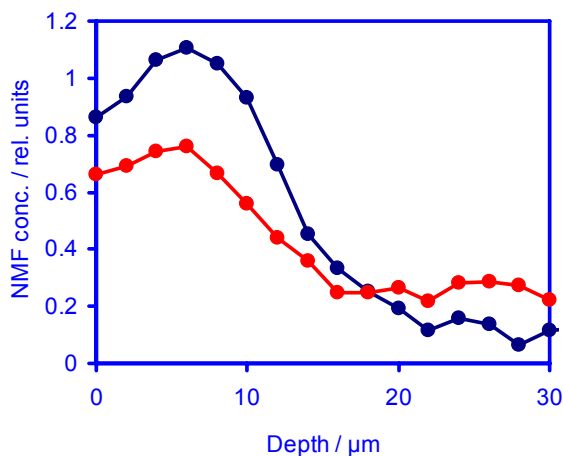


**Figure 2.** Comparison between the composition of human NMF by Raman and *in vitro* methods (skin scraping, tape stripping and water extraction).

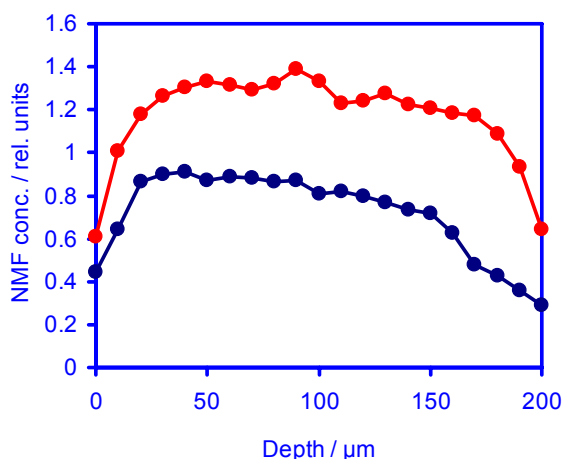
<sup>2</sup> P.J. Caspers, G.W. Lucassen, H.A. Bruining and G.J. Puppels, *Journal of Raman Spectroscopy*, 31 813-818 (2000).

<sup>3</sup> P.J. Caspers, G.W. Lucassen, E.W. Carter, H.A. Bruining and G.J. Puppels, *Journal of Investigative Dermatology*, 116 434-442 (2001).

Figures 3 and 4 show the NMF concentration profiles measured on the forearm and thenar (palm of the hand) of four different volunteers.



**Figure 3.** NMF concentration profile for two volunteers, measured on the volar aspect of the forearm.



**Figure 4.** NMF concentration profile for two volunteers, measured on the thenar.

The NMF concentration is obtained by adding the contributions of the NMF components together.

It is interesting to note that the profiles are different in shape and amplitude for the different volunteers as well as for the different body sites. This is evident in both the differences in absolute amounts of NMF found in different individuals, and also in the maximum depth at which it is found. The latter varies, of course, because the thickness of the stratum corneum varies from individual to individual. These differences are real, as has been demonstrated by repeated measurements.

This fascinating new way of measuring the distribution of molecular species in the skin opens up great possibilities. Not only can static concentration measurements be carried out, but the analysis is fast enough to enable real time *in vivo* monitoring of deposition, diffusion, or chemical conversion of active components in the skin. It will therefore prove invaluable in product development of topically applied cosmetic products and pharmaceutical formulations, and for claims verification as well as for fundamental research in skin science.



**Figure 5.** Marked skin segment on the volar aspect of the forearm of a volunteer. A formulated cream is applied on the skin. No further preparation is needed before measurement with the River Diagnostics model 3510 depth-scanning skin analysis system.

 Instruments for Breakthrough Skin Research

 Partner in Product Development

 Contract Research

 Advancing Knowledge in Skin Science