It was possible to detect hairs by SRS imaging the ear of living mice with a frequency tuned to the protein vibration (CH_3 stretching) (Figure 55.3C).

55.4.2.4 Intraindividual and Interindividual Variability Studied by CRM

Assessing intraindividual and interindividual variability in Raman signatures of components of interest is important for establishing the reproducibility of a CRM protocol across a study population, as well as interpreting possible differences in the penetration kinetics of permeants.

Chrit et al. (2005) studied intraindividual and interindividual variations in Raman spectra by analyzing (1) spectral replicates from one area (the fingertip) of one volunteer; (2) spectra from one volunteer across three body sites (fingertip, thenar or palm, and the volar forearm); (3) spectra from the volar forearm of seven volunteers 28 to 60 years of age; and (4) spectra from the fingertip of a volunteer at different depths into the skin. Replicate spectra from the fingertip of a single volunteer revealed differences in the intensities of the amide I band, as well as a band at 855 cm^{-1} attributed to lactate. The authors suggested a difference in sweat content of the skin yielded this difference. Spectra from the fingertip, thenar, and volar forearm of a single volunteer showed intensity differences in the amide I and amide III regions, as well as for the 855, 880, 935, and 1420 cm^{-1} bands. Further analysis of these spectral differences revealed different ratios of α-helix (1652 cm^{-1}), β-sheet (1660 cm^{-1}), and random coil (1666 cm^{-1}) keratin conformation. Differences between the three regions were also found in lipid conformation (*trans* or ordered vs. gauche or disordered) and in the content of pyrrolidone-5-carboxylic acid, a component of NMF. Comparing the spectra of the forearm of seven volunteers, they found a range of differences in lipids, NMF, and amino acid contents. Cluster analysis depicted that these spectral differences could accurately discriminate the volunteers. Depth-dependent analysis (skin surface to a depth of 48 μm in 12 μm increments) showed differences mainly in the 885 cm^{-1} and 1420 cm^{-1} bands. In the forearm, the water bands in the high wavenumber region (3100 to 3600 cm^{-1}) varied the most.

More recent studies on volunteers have shown overall low interindividual and intraindividual variability, with interindividual variability greater than intraindividual variability. Mogilevych et al. (2015) performed their statistical analysis on four integrated areas of the fingerprint region (996 to 1018 cm^{-1}, 1288 to 1314 cm^{-1}, 1388 to 1498 cm^{-1}, and 1558 to 1722 cm^{-1}). Dos Santos et al. (2016) analyzed the fingerprint and high wavenumber spectra in their entirety, while Quatela et al. (2016) focused their analyses on functional aspects of the skin, i.e., the I_{2880}/I_{2850} intensity ratio (lipid conformation order and lateral packing), the $(I_{1130} + I_{1060})/I_{1085}$ ratio (*trans*/gauche packing), the keratin β-sheet/α-helix ratio, the unbound/partially unbound water ratio, and the maximum in the 2930 cm^{-1} band, an indicator of the folding/unfolding process of proteins. Taken together, the results from these studies lead to the conclusions that confocal Raman spectroscopic data are, to a satisfactory extent, reproducible and comparable. In contrast to Chrit et al. (2005), the populations in these studies covered narrower age intervals (22 to 30, 18 to 37, and 20 to 30 years, respectively), and Raman spectra were acquired only from the volunteers' volar forearms, the most commonly used body site for percutaneous penetration studies. Furthermore, Quatela et al. (2016) showed no significant interday variability over five consecutive days of measurements. A spectral variability study performed by Franzen and Windbergs (2014) using excised human skin from three donors similarly found no statistical interindividual and intraindividual differences.

Another matter of variability is that related to different body sites. As an example, Egawa et al. (2007) determined the water content profiles using the method of Caspers et al. described earlier and deduced the SC thickness of 15 volunteers' cheek, upper arm, volar forearm, back of the hand, and the palm. Body site variation was found, particularly in the water content of the upper SC, which was 30% to 40% at all body sites, except in the palm, where it was 20% to 30%. Differences in SC thickness, in particular that of the palm (mean: 173 μm), were in agreement with results from other methodologies.

A recent work illustrates the use of CRM for a less widely studied epithelium, namely lip skin. Using a dental tongue fixation device, Bielfeldt et al. (2019) developed a novel method for