In vivo measurements on volunteers are most often performed on the volar forearm. RiverD's instruments incorporate a measurement stage containing a fused silica window onto which the volunteer can place his or her forearm. In addition to the forearm, the water composition of the cheek has been investigated by Egawa and Tagami (2008). Pudney et al. (2012) have described a Raman probe allowing investigation of areas of the body that are more difficult to access; e.g., the axilla, the scalp, and the mouth. However, this probe is not currently commercially available.

The 785-nm laser wavelength is generally used to obtain the "fingerprint region" spectra (400 to 1800 cm^{-1}) of the skin; this is also the region within which topically applied compounds are detected. With the RiverD instruments the "high wavenumber region" spectra (2500 to 4000 cm^{-1}) are acquired using a 671-nm laser. As described later, the high wavenumber region spectra are most often used to obtain the keratin and water intensities from which the skin water content–depth profile can be derived. For in vivo use, the RiverD instruments' laser powers are limited to 20 and 30 mW for the 785-nm and 671-nm laser, respectively. Integration times vary from one study to the next but are generally between 1 and 10 seconds. The skin depths investigated in vivo reach from the surface to either 20 or 40 μm into the skin in most studies. The selected depth increment is generally either 2 or 4 μm.

While the RiverD system utilizes two excitation wavelengths to obtain the fingerprint and high wavenumber region spectra, Chrit et al. (2005) demonstrated in vivo confocal spectra using a probe with one laser source (633 nm) that captures the entire frequency region, from 500 to 3600 cm^{-1}.

55.4.2 Tracking Intrinsic Skin Components and Physical Features

Along with the exceedingly complex biology of skin comes its highly complex barrier functions. There are four skin barriers: the physical, chemical, immunological, and microbial skin barrier (Niehues et al. 2018).

CRM has proven highly useful for investigation into the basic makeup and efficacy of the SC barrier. The major intrinsic constituents of skin contributing to and regulating the physical barrier function include water, natural moisturizing factor (NMF), protein (mainly keratin), and lipid compounds (for a comprehensive review, see Dancik et al. (2015)). Interindividual and intraindividual variability of human skin, age, exposure to water or moisturizers, environmental humidity, and dermatological pathologies are factors that may, individually or in concert, alter the amounts and physical organization of these constituents that strongly influence the absorption and penetration kinetics of topical compounds.

55.4.2.1 Total Water Content and Stratum Corneum Thickness

Caspers et al. (1998, 2000, 2001, 2003) showed that water content in the skin of volunteers could be tracked as a function of depth. The methodology they proposed relies on tracking the Raman bands of water and protein and integrating their respective intensities, 3350 to 3550 cm^{-1} (water) and 2910 to 2960 cm^{-1} (protein). The water content is obtained from the ratio of the water mass to the total tissue mass (consisting of water and dry material):

$$Water\ content = 100\% \times \frac{W}{P} / \left(\frac{W}{P} + R \right)$$

with W and P designating the water and protein integrated intensities and R a fixed water-to-protein signal proportionality constant. A typical water content–depth profile is shown in Figure 55.1. The Raman spectra were collected at different depths from the skin surface (0 μm) to 20 μm. The intensity of the stretching band of the O–H bond of water increases with depth into the skin. A sharp increase of the Raman band occurs when passing from the SC to the viable epidermis because the viable epidermis contains much more water (~70%) than SC. The water content at the skin surface is typically around 30%. Going deeper into the SC, the water content increases until a plateau value