55.4.2.2 Types of Water Molecules

The general water band in the high wavenumber region is made up of a number of sub-bands assigned to differently bound water molecules. Vyumvuhore et al. (2013, 2015) showed that deconvolution of the total water Raman band (3100 to 3700 cm^{-1}) using its second derivative yielded sub-bands assigned to totally (primary) bound water (3210 cm^{-1}), partially bound water (3280 and 3345 cm^{-1}), and unbound or free water molecules (3470 cm^{-1}). Boireau-Adamezyk et al. (2014) also split the total water signal into three subtypes using a perpendicular drop-down cutoff integrating method, i.e., subdividing the total water band into three regions assigned to "bound," "intermediately mobile," and "mostly mobile" water types (Figure 55.2). The separation into several types refers to the state of binding of water molecules to neighboring protein and lipid constituents of the SC. Totally bound water molecules are tightly bound to the polar sites of SC protein; partially bound water molecules interact through only two or three out of the four hydrogen bonds, whereas unbound water molecules are not directly linked to other SC components.

In a series of papers, Darvin and Lademann's group illustrated the use of a methodology for studying the distribution of water types as a function of depth in the SC (Choe et al. 2016, 2018a, 2018b; Sdobnov et al. 2019). Arguing for greater precision than provided by the perpendicular drop-down cutoff integrating method, they deconvoluted the entire high wavenumber spectral region using 10 Gaussian functions. Among the obtained sub-bands, four are assigned to the different water types: the tightly hydrogen-bound water molecules (single donor–double acceptor around 3005 cm^{-1}), the strongly hydrogen-bound water (double donor–double acceptor around 3277 cm^{-1}), the weakly hydrogen-bound water (single donor–single acceptor around 3458 cm^{-1}), and the free water.

Identifying and tracking the water types is important for the characterization of water mobility in SC and delivery of topical compounds to skin (Boireau-Adamezyk et al. 2014) as well as differentiating between healthy and diseased skin (Vyumvuhore et al. 2013). Binding of water determines the flexibility and stiffness of the SC. Healthy skin contains about 33% of bound water (g water per 100 g tissue) (Vyumvuhore et al. 2013).

Direct measurement of water penetration in the skin by CRM requires replacing water of natural isotopic abundance by deuterated water, D_2O, which shows a Raman peak corresponding to the O–D stretching vibration at 2500 cm^{-1}, different from endogenous water and clearly distinguishable

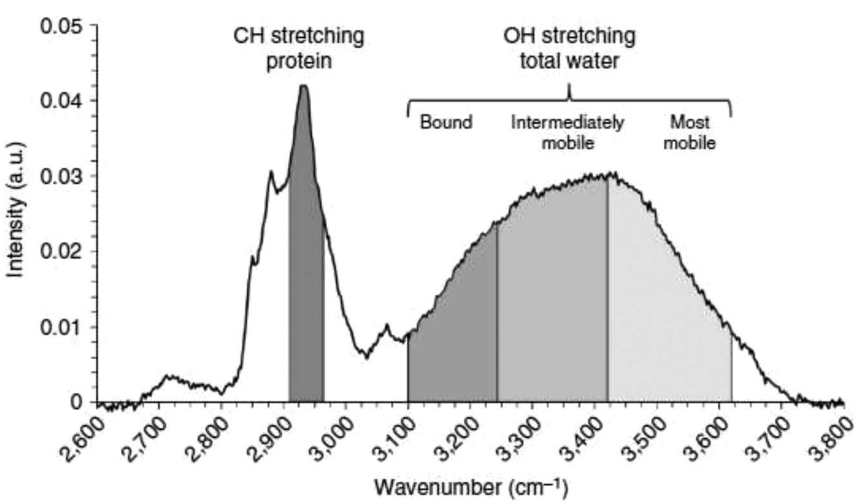

FIGURE 55.2 Raman confocal spectra in the high wavenumber region highlighting the bands of CH stretching and OH stretching that can be used for the calculation of water content and that of the water molecules in the three states based on their mobility. (Reproduced from Boireau-Adamezyk et al. 2014 with permission.)