in the spectra (Förster et al. 2011b). By applying emulsions and surfactant solutions made in deuterated water, the authors observed that water penetrated from the formulations into the SC, keeping a profile similar to that of endogenous water. Penetration of deuterated water in the SC after application of pure D_2O on skin under occlusive conditions was confirmed by Ashtikara et al. (2013).

55.4.2.3 Lipid Conformation and Lateral Packing

CRM has been proven useful in making fundamental observations related to lipid conformation and lateral packing order (Choe et al. 2016; Vyumvuhore et al. 2013, 2015).

Different Raman spectral manifestations of the conformational order of lipids can be drawn from experiments performed on thin films of lipids, such as in recent in vitro studies on ceramide films prepared from seven classes and subclasses of ceramides (Tfayli et al. 2010, 2012a). For example, intrachain conformational order information is obtained from the bands of C–C skeletal optical mode and the band of the twisting CH_2 group, whereas the CH_2 scissoring and stretching modes reflect the lateral packing of the lipid molecules, and the amide I band is used to evaluate the strength of H-bonds (Tfayli et al. 2012a). In vivo determination of lipids ordering by Raman spectroscopy is much more difficult (Tfayli et al. 2012b).

A nice example of in vivo skin imaging used SRS microscopy (**Section 3**) was provided by Saar et al. (2010). In vivo skin optical imaging of mouse skin was achieved by focusing on three vibration bands to image skin structure: lipids (CH_2 stretching, 2845 cm^{-1}), water (OH stretching, 3250 cm^{-1}) and proteins (CH_3 stretching, 2950 cm^{-1}). This allowed imaging the water, lipid, and protein distribution of the SC and the viable epidermis (Figure 55.3A and B). The corneocytes and the intercellular spaces clearly appeared, as well as the sebaceous glands in the epidermis, which appeared both in lipid and water images, i.e., when the incident laser frequency was tuned to the lipids (CH_2 stretching) or water (OH stretching) vibrations. Sebaceous glands appeared clear when the frequency was tuned to that of lipid vibration and dark when it was tuned to the water vibration.

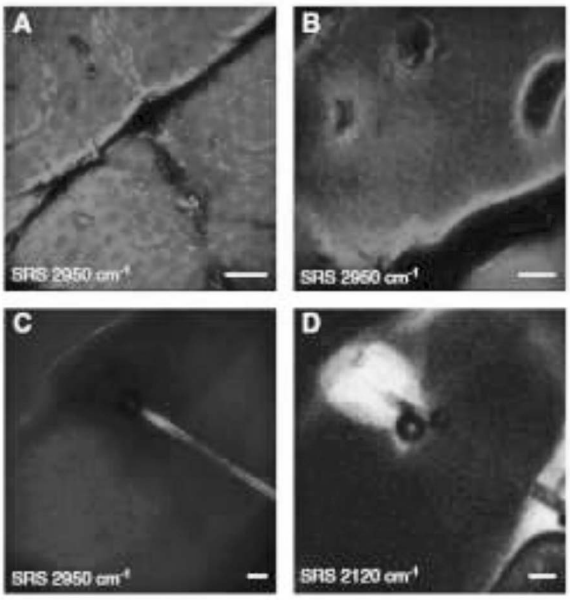

FIGURE 55.3 SRS skin imaging of the forearm of a volunteer; A to C: the frequency was tuned to the CH_3 stretching vibration of proteins at 2950 cm^{-1}: (A): stratum corneum; (B) viable epidermis; (C) hair shaft; (D) SRS skin imaging after applying deuterated DMSO-d_6 (the frequency was tuned to the specific vibration of DMSO-d_6 2120 cm^{-1}) in the same region as shown in (C).