55.3 VARIANTS OF RAMAN SPECTROSCOPY

There are several variants of classical Raman spectroscopy besides the spontaneous Raman scattering technique where the spectrum acquisition is a direct measurement of the scattered light. Surface-enhanced Raman scattering (SERS) shows enhanced intensity for molecules located in the vicinity of metal particles. The sensitivity improvement requires the deposition of metal nanoparticles at the surface of the sample. SERS allows a specific analysis of the species present at the sample surface. Resonance Raman spectroscopy provides a selective spectrum of a molecule with high sensitivity when the frequency of the laser light of the Raman spectrometer is tuned to a UV-vis absorption band of the molecule. Coherent Raman spectroscopy (Min et al. 2011) relies on non-linear effects of two-photon absorption. A resonance occurs when the frequency difference between the two lights matches the frequency of the molecular vibration. A signal-to-noise ratio enhancement by several orders of magnitude is achieved when the resonance condition is met. However, such resonance methods are not spectroscopic methods because a single Raman frequency is measured at once. Coherent anti-Stokes Raman scattering (CARS) (Evans and Xie 2008) and stimulated Raman scattering (SRS) (Downes and Elfick 2010; Slipchenko et al. 2010) are the two major techniques. Several improvements of these techniques were recently introduced in order to increase the sensitivity an decrease the non-resonant background by epi-detection (Li et al. 2005); high-speed confocal microscopy data can be collected (Saar et al. 2010). Equipment for two-color microscopy has been designed (Lu et al. 2012). SRS appears more sensitive and accurate than CARS (Nandakumar et al. 2009; Freudiger et al. 2008). Such techniques allow time-resolved confocal microscopy measurements with high sensitivity at the selected vibration frequencies of the penetrant molecules. Determination of band shifts that are characteristic of interactions of penetrant molecules and skin components requires several measurements at different specific frequencies and merging them into a full spectrum. A recent SRS technique making use of modulation of excitation Raman frequencies and signal demodulation by Fourier transform allows the acquisition of several Raman bands at the same time, and possibly parts of the Raman spectra (Fu et al. 2012). SRS has been used to investigate the lipid distribution in skin (Klossek et al., 2017; Wanjiku et al., 2019), as well as the skin absorption of *trans*-retinol and propylene glycol (Saar et al. 2010), ketoprofen and ibuprofen (Saar et al. 2011), and retinoic acid (Freudiger et al. 2008). Near-infrared Fourier transform (NIRFT) Raman spectroscopy designates the use of a higher wavelength laser, typically 1064 nm, to probe skin (Barry et al. 1992; Williams et al. 1993; Anigbogu et al. 1995; Gniadecka et al. 1998; Schallreuter et al. 1999; Wohlrab et al., 2001; Knudsen et al. 2002; William et al. 2004; Naito et al. 2008). Advantages over lower, more conventional wavelengths such as 785 nm are deeper penetration of the incident light into the tissue and reduced signal attenuation due to scattering. However, in most NIR systems the laser powers are in the range of a few hundred mW, which may be unsafe for volunteers. Several NIR studies with volunteers have nonetheless been conducted (Knudsen et al. 2002; Schallreuter et al. 1999; Naito et al. 2008).

55.4 CHARACTERIZATION OF INTRINSIC HUMAN SKIN PARAMETERS RELATED TO DRUG PENETRATION

55.4.1 INSTRUMENTATION AND SETTINGS FOR STUDIES WITH VOLUNTEERS

Thanks to the non-invasiveness and non-destructiveness of the method, Raman spectroscopy lends itself to in vivo characterization of the basic composition and morphology of human skin, as well as tracking chemicals applied onto the skin and studying the ensuing effects on skin physiology and morphology. Measurements on volunteers require a Raman spectroscope certified for use with volunteers. Most published studies make use of the gen2-Skin Composition Analyzer (SCA), or its predecessor, the Model 3510 SCA, from RiverD International (Rotterdam, The Netherlands).