In 2018, Banzhaf et al. conducted an explorative experiment to determine whether the combined technologies of RCM and fluorescence confocal microscopy could provide information on drug uptake, distribution, and kinetics in ablative fractional laser-exposed skin [27]. Thawed human skin was used as a model, and sodium fluorescein in a prototypical gel formulation was applied to the skin as a surrogate hydrophilic drug as well as for visualization. Ablative fractional laser was applied to the skin and then the gel was gently massaged into that area. RCM and fluorescence confocal microscopy images were taken at 15 minutes, 60 minutes, and 4 hours. RCM images revealing the morphology of the skin strata (stratum corneum, epidermis, and upper dermis) were recorded at every second micron from the skin surface to a depth of 200 µm (Figure 56.15, left). Fluorescence confocal microscopy images provided a fluorescein signal from the gel. Merged images of RCM and fluorescence confocal microscopy indicated that the ablative fractional laser created channels in the skin, creating a coagulation zone that penetrated to the upper dermis. RCM and fluorescence confocal microscope z-stacks were analyzed with ImageJ to assess the fluorescein delivery according to the skin depth (Figure 56.15, right). RCM has a limitation of decreasing resolution in deeper skin layers; however, this proved for the first time that using both RCM and fluorescence confocal microscopy were useful to investigate uptake, biodistribution, and kinetics of a test drug in ablative fractional laser-treated skin.

Labouta et al. (2011) measured the penetration and metabolic effects of AuNPs in aqueous and toluene solutions on excised human skin [28]. They used both frozen and freshly excised human skin for multiple imaging techniques, including RCM, TEM, MPT, and FLIM. The freshly excised

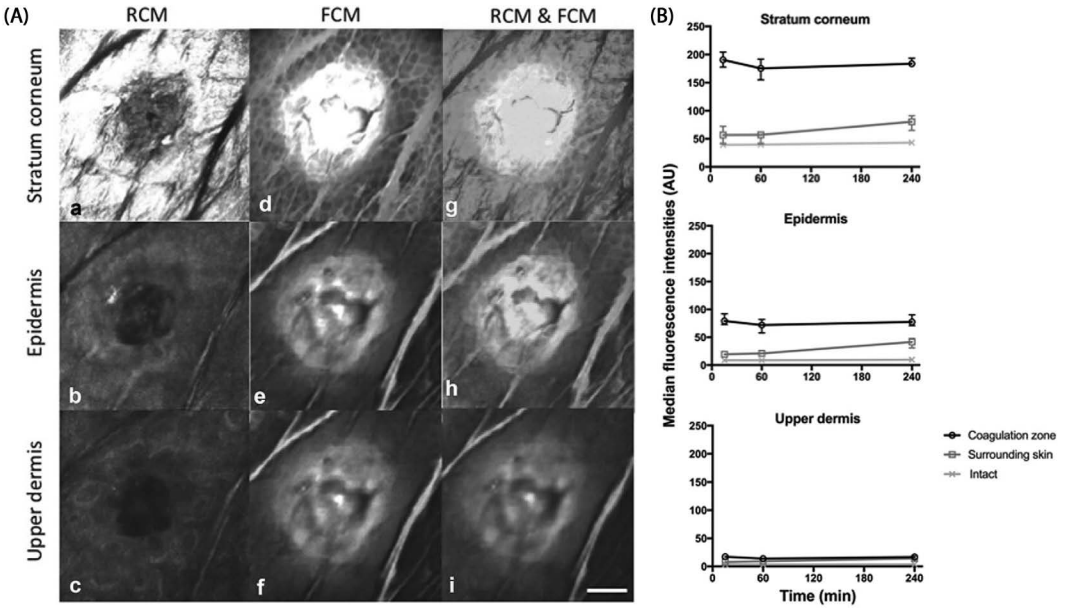

FIGURE 56.15 (A) Laser scanning confocal microscopy images from specific skin compartments after AFXL exposure and sodium fluorescein (NaF) application. (a–c) Representative reflectance confocal microscopy (RCM) images of a laser channel, including coagulation zone (CZ) at the stratum corneum, epidermal, and dermal level and (e and f) corresponding fluorescence confocal microscopy (FCM) images. (g–i) Merged red-green-blue (RGB) channels of RCM and FCM images illustrating the distribution of NaF in CZ and surrounding skin throughout the skin compartments (scale bar 100 µm). (B) Fluorescence intensities (Fl) of sodium fluorescein (NaF) in the stratum corneum, epidermis, and upper dermis after 15 minutes, 60 minutes, and 4 hours, respectively. The highest FI occurs in the coagulation zone (CZ) in the stratum corneum and epidermis. FI from surrounding skin at four hours is increased in the epidermis and upper dermis, suggesting enhanced delivery of NaF ($P = 0.03$). (Reprinted with permission from Banzhaf et al. 2017.)