Many studies have used fluorescent-labeled drugs. Tracing fluorescence, assuming the drug is firmly attached, has been the most common approach for the assessment of transdermal drug delivery [1, 2]. Fluorescence-based microscopes range from affordable fluorescence microscopy to expensive, sophisticated equipment, such as multiphoton or fluorescent-lifetime imaging microscopy (FLIM), has been discussed [3, 4]. Image analysis is often done by fluorescent signal intensity plotted against skin depth. A limitation of fluorescent-based microscopy is autofluorescence of the skin. More advanced microscopy approaches such as multiphoton or FLIM can better differentiate the signal of active molecules from skin autofluorescence by counting photons from each fluorophore lifetime. Another limitation of fluorescence-based microscopy is that dye labeling can change the molecular structure of the drugs and consequently might influence the penetration profiles. In addition, histologically prepared samples or ex vivo samples may show different drug penetration profiles compared to in vivo samples. The penetration process of nonfluorescent substances can be analyzed by Raman spectroscopy and electron microscopy measurements. Using these methods, semiquantitative analysis of actives in different depths of the skin can be detected by moving the laser focus from the skin surface into deeper layers. However, despite the high spatial resolution of these methods, the sensitivity and chemical specificity are limited, which leads to uncertainty regarding the interpretation of the images. The latest advancement in the analysis of drug penetration assessment is matrix-assisted laser desorption/ionization (MALDI) mass spectrometry imaging (MSI). This approach allows imaging of a tissue sample whereby the laser is rastered across the sample [5]. Mass spectra are then collected for each pixel. The distribution of the molecules within the tissue samples can be presented as a 2D image, with pixel intensity related to the abundance of a particular mass peak. MALDI mass spectrometry can also detect specific drug molecules and metabolites by using an accurate mass measurement within biological samples, which gives more accurate quantification of drugs compared to any of the other techniques mentioned so far. This technology would be greatly beneficial in a clinical setting. At present, a reflectance confocal microscope (RCM) is being used in volunteer studies related to percutaneous drug penetration [6]. RCM is increasingly being used as a noninvasive adjunctive tool in dermatology. With RCM, penetration can be viewed in horizontal sections of skin with resolution comparable to histology, observed drug affects in living skin, and monitored treated area longitudinally if the active has the requisite reflectance properties [7, 8]. A summary of advantages and disadvantages of each microscopy technique in topical drug penetration studies is shown in Table 56.1.

A range of microscopic methods have been used to evaluate cutaneous absorption of drugs in the development of novel formulations to be administered through the skin. Table 56.2 gives an overview of actives that have been applied to the skin and the methodologies used to determine penetration that appear in the recent literature. Selecting the right model for each active ingredient is important to assess topical drug penetration. The cost, requirement for training, and availability of equipment are other important factors to consider when selecting an imaging methodology. We will now describe each of the current imaging technologies and give examples of microscopy-based approaches that have been used for cutaneous drug delivery assessments.

56.2 FLUORESCENCE MICROSCOPY

A fluorescence microscope is an optical microscope that reveals fluorescence emission. The term refers to any microscope that uses fluorescence to generate an image, including epifluorescence microscopes or more complicated design such as confocal microscopes, which use optical sectioning to get a better resolution. Topical drugs that are labeled with fluorescence can be traced by a fluorescence microscope. It enables users to directly visualize and quantify fluorescence drugs within the skin. Skin autofluorescence is a major limitation of this technology, which makes straightforward identification of specific drugs challenging.

Lee et al. (2015) used fluorescent images to assess the penetration of rhodamine-labeled human beta-defensins (hBDs), using nude mice skin ex vivo [9]. hBDs are crucial factors of intrinsic