TABLE 56.2 (*Continued*)
List of Targets that Have Been Studied by Each Microscopic Methodology

Methodology	Targets	Model	References
	Sodium fluorescein	In vivo (human)	[29]
	Gold nanoparticles	In vivo (human)	[6]
	Garcinia mangostana	In vivo (human)	[58]
MALDI-MSI	Roflumilast, tofacitinib, ruxolitinib	Ex vivo (human)	[29]
	Bleomycin	In vivo (human)	[30]
TOF-SIMS	Ceramide	Ex vivo (human)	[31]
	Liposomal doxorubicin	Ex vivo (pig)	[59]
		Ex vivo (pig)	
		Ex vivo (pig)	

immunity against a variety of invading enveloped viruses, bacteria, and fungi. Three different hBD peptides were synthesized (hBD3-1, hBD3-2, and hBD3-3) and labeled with rhodamine 123 (peak excitation wavelength = 507 nm). The rhodamine-labeled peptides were applied in separate locations on the back of the neck in mice. After three hours of treatment, the skin was collected and embedded for frozen sections. The skin tissues were sectioned and placed onto histological slides for imaging. Fluorescence microscopic images show positive rhodamine (emission = 575 nm, Figure 56.3 [9]). hBD3-3 shows a strong signal throughout the epidermis, whereas hBD3-1 and hBD3-2 show fluorescent staining of the stratum corneum. These results were consistent with the in vitro (macrophages) cell penetration of peptides. The skin autofluorescence creates a strong background in images, which makes it hard to define the true signal from rhodamine. Another problem was that the low resolution of images from each skin sample, which illustrates one of the limits of this approach. This limits the information on the drug penetration profile. There was no quantitative analysis in this study. Overall, the fluorescence microscopy approach used in this study reveals the utility and feasibility of visualizing a situation where there is minimum transdermal peptide delivery in the skin.

Smejkalova' et al. (2017) [10] assessed cosmetically relevant active hyaluronic acid (HA) penetration in pig skin using fluorescence microscopy. HA is one of the most hydrophilic molecules in nature. They made hydrophobic HA using derivatives and formulated these into polymeric micelles loaded with cosmetic active coenzyme Q10 (C). Nile red (excitation = 558 nm) was encapsulated within the core of the micelles for visualization. They tested other emulsion formulations as controls. They applied these formulations on excised pig skin. After 20 hours, they cryomoulded the tissue and sectioned it onto histology slides. Images were taken by fluorescence microscopy (Figure 56.1). Nile red was detected in the skin after 20 hours of treatment. The skin orientation and location were confirmed with the same image under phase contrast. The fluorescent images showed that delivery of the hydrophobic compounds in HAC6 and HAC18 polymeric micelles were enhanced by hydrophobic HA. The conclusion from the imaging data was that the more hydrophobic the drug becomes (HAC18), the deeper the dye can penetrate into the dermis. The sections were later homogenized to extract Nile red for a relative quantification (T = 5 hours and T = 20 hours) (Figure 56.1, bottom graph). The quantitative analysis results supported the visual data from fluorescent imaging data. The imaging data were limited by the lack of quantitative measurements, sample preparation for frozen sectioning could create artifacts, and ice crystal formation may interfere with polymeric micelles structure, as well as its delivery profile into the skin. The staining process (physical washing or PBS for washing solution) may also affect the delivery profile of the polymeric micelles. This study is a relevant example of visual assessment of cutaneous drug penetration using cross-section imaging with fluorescent microscopy.