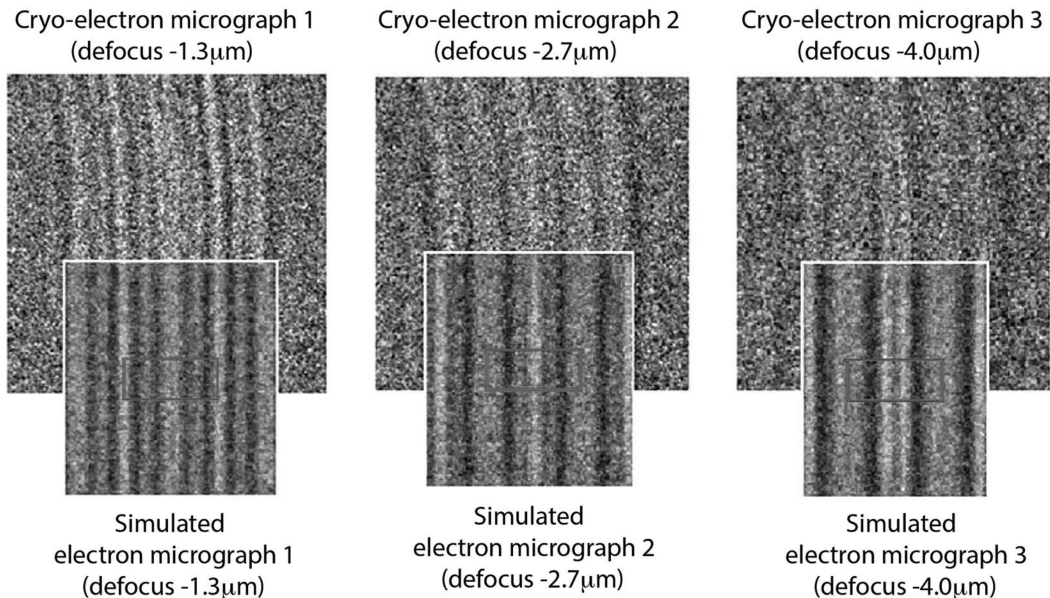

FIGURE 1.3 CEMOVIS micrograph defocus series from human skin (upper/back row) compared with simulated electron micrograph defocus series from the atomistic MD model in Figure 1.2. Upper/back row: Sequential CEMOVIS micrograph defocus series recorded at –1.3 µm, –2.7 µm, and –4.0 µm defocus. Lower/front row: Simulated sequential electron microscopy defocus series recorded at –1.3 µm, –2.7 µm, and –4.0 µm defocus. It is shown that the atomistic MD model in Figure 1.2 accurately accounts not only for the skin's CEMOVIS micrograph intensity patterns at a single defocus but also for the intensity pattern changes observed upon varying the microscope's defocus during image acquisition. Pixel size in the CEMOVIS and in the simulated electron micrographs: 1.88 Å. (Adapted from Iwai et al. 2012 and Lundborg et al. 2018a, respectively, with permission.)

with, e.g., multiple local maxima for the permeation of water as a function of model system water content (Lundborg et al., 2018a,b).

1.5 CEMOVIS

With CEMOVIS, the native tissue is preserved down to the molecular level, and the micrograph pixel intensity is directly related to the local electron density of the specimen (Dubochet et al., 1988; Al-Amoudi et al., 2004; Norlén et al., 2009). As biomolecules are essentially composed of atoms with similar atomic weight (carbon, nitrogen, and oxygen), they generally possess small intermolecular and intramolecular differences in electron density. However, for orderly arranged molecular assemblies, such as lipid headgroups and tails in membranes, even small differences in shape and atomic composition may be amplified due to interference effects that appear in the image phase contrast. During cryo-EM image acquisition, phase contrast is made visible using defocus (cf. e.g., Fanelli and Öktem, 2008).

At very high magnification (pixel sizes of a few Ångström), complex interference patterns can be resolved in CEMOVIS micrographs (Iwai et al., 2012). This can be exploited by recording CEMOVIS micrographs repeatedly at the same position of the tissue sample while increasing stepwise the microscope's defocus, ensuring that differences in the recorded micrographs are exclusively due to the different defocuses used. Thus, the recorded CEMOVIS defocus series images represent a range of unique phase contrast patterns for the underlying biological structure, which can be used for structure determination (Iwai et al., 2012; Lundborg et al., 2018a) (Figure 1.3, upper/back row).