Data S1

eMPM findings. In one LS patient, images were first acquired with RCM *in vivo* and then a skin biopsy was obtained from the area investigated with RCM. This biopsy was then investigated with MPM *ex vivo*.

An LSM 710 NLO microscope system (Carl Zeiss MicroImaging GmbH, Germany) was used for MPM imaging. The optical resolution was 0.5 μ m lateral and 1.5 μ m axial. An InSight x3 laser (Spectra-Physics, Newport Corporation, USA) was used for excitation. The excitation wavelength was set to 780 nm to target autofluorescence, and the power

setting was optimized to yield a similar fluorescence signal. Fluorescence from the tissue was collected in the emission range of 410-690 nm using a non-descanned, highly sensitive GaAsP detector.

The fiber-like structures in the papillary dermis representing hyaline sclerosis and the typical honeycomb pattern were visualized with both techniques (Fig. S7). Nevertheless, the fiber structures were brighter and sharper in the MPM. Thus, the data from this patient support that hyaline sclerosis can be visualized better with MPM *ex vivo* than with RCM *in vivo*. Figure S1. Reflectance confocal microscopy data acquired from (A) 1 patient with LS, (B) 1 individual with healthy penile skin, (C) 1 patient with nonspecific balanoposthitis, (D) 1 patient with plasma cell balanitis and (E) 1 patient with PeIN in the level of stratum spinosum. A typical honeycomb pattern (blue circles) was observed in (A) LS, (B) healthy penile skin, (C) nonspecific balanoposthitis and (D) plasma cell balanitis, (E) whereas an atypical honeycomb pattern (red circles) was seen in PeIN. The typical honeycomb pattern corresponds to the normal epidermal architecture, whereas the atypical honeycomb pattern corresponds to the disarrayed epidermis architecture found in squamous cell carcinoma *in situ*. Size of images, 0.5x0.5 mm. Scale bar, 100 μ m. LS, lichen sclerosus; PeIN, penile intraepitelial neoplasia.



Figure S2. Reflectance confocal microscopy data acquired from 1 patient with lichen sclerosus with simultaneous penile intraepitelial neoplasia. Scattered, small, bright cells (blue arrow) are seen in the stratum basale, which may correspond to the cell atypia and hyperchromacy. Size of image, 0.5x0.5 mm. Scale bar, 100 μ m.



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Figure S3. Reflectance confocal microscopy data acquired at the level of the papillary dermis from (A) 1 patient with LS, (B) 1 patient with nonspecific balanoposthitis and (C) 1 individual with healthy penile skin. As is illustrated by the figure, bright areas (blue circles) corresponding to the dermal inflammatory infiltrate were found in LS and nonspecific balanitis. However, this feature was not seen in the healthy skin. Size of images, 0.5x0.5 mm. Scale bar, 100 μ m. LS, lichen sclerosus.



Figure S4. Reflectance confocal microscopy data acquired from a patient with nonspecific balanoposthitis at the level of stratum basale. Refractive cells were seen (red asterisks) and bright cells flowing inside linear, canalicular structures in the black lumen of the papillae (blue arrow). The refractive cells represent inflammatory cells, and the linear structures represent dilated vessels. Image size, 0.5x0.5 mm. Scale bar, 100 μ m.



Figure S5. Reflectance confocal microscopy data acquired from a patient with plasma cell balanitis taken at the stratum spinosum layer. As can be seen, mildly refractive cells were observed in the intercellular spaces (exocytosis) between keratinocytes (blue arrows). This feature is associated with spongiosis. Image size, 0.5x0.5 mm. Scale bar, 100 μ m.



Figure S6. Reflectance confocal microscopy data acquired at the level of the papillary dermis from (A) 1 patient with lichen sclerosus, (B) 1 patient with balanoposthitis, (C) 1 patient with plasma cell balanitis and (D) 1 individual with healthy penile skin. Irregular papillae in terms of their shape (blue arrows) were observed in all images. Image size, 0.5x0.5 mm. Scale bar, 100 μ m.



Figure S7. (A) Reflectance confocal microscopy data compared with (B) MPM Data acquired from the same area in 1 patient with lichen sclerosus at the level of the papillary dermis. As can be seen, the fiber-like structures representing the sclerosis were visualized with both microscopes (blue arrows). However, they were brighter and sharper for MPM. Size of images, 0.5x0.5 mm. Scale bar, 100 μ m. MPM, multiphoton microscopy.

