Appendix S1. Stromal boundaries

To standardize backscatter measurement of the corneal stroma, we first described a clear and repeatable method that defined the posterior and anterior boundaries of the stroma. We defined these boundaries on the basis of the IVCM images. Because specular reflection of the endothelium and backscatter of Descemet’s membrane and Bowman’s layer may affect mean stromal backscatter, we used a margin of safety of 12 µm (two images) on both sides of the stroma. Figure 1 and 2 illustrate our methods to derive both boundaries. The frame numbers corresponding with the images of the stromal boundaries were noted for all complete passes in a single scan.

Figure 1. Posterior boundary of the corneal stroma. Considering the IVCM images in their original order from endothelium to epithelium, we located the first image that showed the keratocyte nuclei without any visible details of the endothelial layer (asterix). We then selected the image two frames further into the stroma (double asterix). This image represented the boundary of the posterior stroma of which the frame number within brackets (circle) was noted.
Figure 2. Anterior boundary of the corneal stroma. Similar to the method for determining the posterior boundary, we considered the IVCM images from endothelium to epithelium. The most anterior image showing the keratocytes without any visible details of the subbasal nerve plexus (asterix) was located. Then the image two frames back into the stroma was selected (double asterix). For this image, which represented the anterior stromal boundary, the frame number within brackets (circle) was noted.