Multiphoton Imaging of the Cornea. Basic Science and Clinical Applications.

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Multiphoton (MP) imaging using femtosecond lasers provides a non-invasive means of imaging the structural organization of the cornea through the generation of second harmonic signals (SHG) from collagen. To map the collagen organization within the eye we have used computed tomography on optical and mechanical sectioned tissue to generate high-resolution macroscopic (HRMac) images that cover the entire tissue. 3-Dimensional tomographs varied in size from 9 to 90 Meg pixels per plane, had a pixel resolution of 0.44 mm lateral and 2.0 mm axial, and covered regions extending from 9 mm^2 to 100 mm^2. 3-D reconstructions revealed a complex collagen fiber branching pattern in the anterior cornea with fibers extending from the anterior limiting lamina (ALL), intertwining with deeper fibers and then reinserting back to the ALL forming 'bowspring-like' structures. Fibers were also identified that extended from the limbus and traversed across large portions of the cornea and then inserted into the ALL. Measured branching point density was four times higher in the anterior third of the cornea compared to the posterior third and decreased logarithmically with increasing distance from the ALL. Compression testing showed increased stiffness in the anterior part of the stroma. These reconstructions allowed for regional measurements within the cornea, orientation and organization over the entire tissue that could be directly correlated to tissue stiffness using biomechanical testing. These results indicate that the axial gradient in lamellar intertwining is associated with an axial gradient in effective elastic modulus of the cornea that may control shape and function.