Orientation of collagen fibers in tendons by second harmonic generation microscopy

P. Bianchini¹, G. Vicidomini¹, J. Janâcek², J. Kubinová², A. Diaspro¹
¹ LAMBS, MicroSCoBiO University of Genoa, Genoa, Italy
² Department of biomathematics, Inst. of Physiology, Academy of Sciences of Czech Republic, Prague, Czech Republic

KEYWORDS: SHIM, SHG, TPEF, fluorescence, confocal microscopy, non linear optics.

It’s known that several endogenous protein structure give rise to second-harmonic generation (SHG) – second order nonabsorbative energy doubling of an excitation laser line [1].

The orientation of collagen fibers within tissues such as tendons or ligaments is of primary importance. In this study, we propose a simple method based on second harmonic generation (SHG) microscopy to map, pixel by pixel, the orientation of the symmetry axis of the second-order nonlinear susceptibility tensor of collagen fibers of a tendon. The method uses only four images acquired at specific polarizations of the input laser beam by rotating the sample on the stage. In addition to orientation information, the method would provide polarization independent images and an estimation of the ratio of the nonlinear susceptibility components. This procedure is implemented in both backward and forward scattering pathway [2]. By correlating the mean orientation of the nonlinear susceptibility to the fibril orientation itself for many fibril segments seems that both orientations are truly parallel at the fibril scale. This approach could allow to map fiber orientation fields, independently of individual fiber contrast in the SHG image. The comparison between the resulting images acquired in forward and backward scattering should tell more about the comprehension of the backscattered signal nature.
