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Interferometric Second Harmonic Generation imaging of biological tissues

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Second harmonic generation (SHG) microscopy is used to image structures lacking a center of inversion (non-centrosymmetric). Some biological tissues, particularly those made of collagen, possess this property. Because SHG microscopy is a coherent technique, the structure of the studied sample influences greatly the generated signal strength. By varying the polarization of the fundamental beam, the different $\chi^{(2)}$ elements of the sample can be measured and related to this structure. Hence, general alignment and organization of a tissue can be evaluated.

However, the phase of the signal is lost in the measurement. In interferometric SHG (I-SHG) imaging, second harmonic is generated a first time out of the microscope. This signal is then directed towards the imaging setup where it interferes with the second harmonic generated in the tissue. By varying the relative phase between the two second harmonic signals that are interfering, the relative phase of the $\chi^{(2)}$ in the tissue can be retrieved pixel by pixel. We have used this technique to image myosin filaments in muscles, tendon, fascia and cartilage. This technique can help to understand the structure of these tissues at the 100 μm level and relate it to its function in the human body and its evolution relative to disease and aging.

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