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Interferometric second harmonic generation imaging of biological tissues

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In recent years, Second Harmonic Generation (SHG) microscopy has emerged as a powerful technique to image in situ non-centrosymmetric structures in biological tissues, such as collagen, a major structural protein of vertebrates. However, due to the coherence of SHG signal, intensity depends not only on the density and the overall organization of harmonophores, but also on their relative polarity within the focal volume. In SHG imaging, the phase of the signal, which contains this additional structural information, is lost in the measurements which has impeded up to now the study of the nanoscale arrangement of the fibrils' polarity.

To overcome this limitation we recently, proposed Interferometric SHG (I-SHG) microscopy. In I-SHG microscopy, second harmonic is generated twice: first outside the microscope, to provide a reference SHG beam, and second within the sample, to probe the fibrils'polarity. By varying the phase between the two beams that are interfering, the relative phase of the second-order susceptibility, or equivalently the relative orientation of the second harmonic emitters, can be retrieved pixel by pixel. As a proof of concept we first applied I-SHG to the study of myosin filaments in muscles and highly organized collagen bundles in tendons. However, the investigation of tissues exhibiting more complex collagen architecture, such as cartilage, has been hindered so far by the low interferometric contrast obtained with picosecond pulses laser. We therefore implemented for the first time I-SHG using femtosecond pulses and applied it to study the collagen meshwork in cartilage. To do so, we developed a collinear setup to compensate for the dispersion introduced in the microscope as to optimize both spatial and temporal overlap.

The results are analyzed in regards of numerical simulations showing that the phase measurements can be related to the local ratio of fibrils with opposite polarities, which is responsible for the strong variations of signal intensity in our images. A comparison with standard and polarization-resolved SHG highlights the role of both tissue organization and relative fibril polarity in determining the SHG signal intensity. This work illustrates how the complex architecture of non-centrosymmetric scatterers at the nanoscale governs the coherent building of SHG within the focal volume and the observed features in SHG images.

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