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# Interferometric Second Harmonic Generation Microscopy and applications. (I)

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Over the years, Second Harmonic Generation (SHG) microscopy has emerged as an effective tool in biology. Like in two-photon excited fluorescence (2P), this type of laser scanning microscopy is characterized by an intrinsic 3D sub-micron resolution that is robust upon light scattering and which allows for higher image depth when compared to confocal microscopy. SHG is a nonlinear optical process in which highly polarizable and non-centrosymmetric structures emit photons at exactly half the excitation wavelength. The emitted light results from the coherent sum of the electromagnetic field generated by every single SHG emitter and thus scales quadratically with the number of aligned molecules sharing the same polarity. Indeed, adjacent molecules of the same polarity will emit strong SHG signals due to constructive interference while the SHG signal will almost vanish in the case of adjacent molecules of opposite polarity. Furthermore, by measuring the square of the SHG amplitude, structures of opposite polarity cannot be distinguish using this imaging technique.

Originally developed to characterize non-centrosymmetric crystals, interferometric SHG (I-SHG) microscopy is based on the measurement of the phase of the SHG signal. In the past years, its potential for tissue imaging has been demonstrated with different proteins, such as myosin from skeletal muscle [1] and collagen from tendon and cartilage [2,3]. Having recently solved one of the main drawbacks of I-SHG, namely the long imaging time, we have recently demonstrated the possibility to use I-SHG to record the dynamical evolution of microtubule polarity in mitotic spindles from live zebrafish embryos [4].

[1] Rivard, M. et al. Imaging the bipolarity of myosin filaments with interferometric second harmonic generation microscopy. Biomed. Opt. Express **4**, 2078–2086 (2013).
[2] Rivard, M. et al. Imaging the noncentrosymmetric structural organization of tendon with interferometric second harmonic generation microscopy. J. Biophotonics **7**, 638–646 (2013).
[3] Couture, C.-A. et al. The impact of collagen fibril polarity on second harmonic generation microscopy. Biophys. J. **109**, 2501–2510 (2015).
[4] Bancelin, S. et al. Probing microtubules polarity in mitotic spindles in situ using Interferometric Second Harmonic Generation Microscopy. Nature Sci. Rep. **7**, 6758 (2017).

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