FAST POLARIZED THG MICROSCOPY OF ANISOTROPIC BIOMATERIALS

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Polariezd third-harmonic generation (P-THG) is a sensitive probe of materials anisotropy and molecular ordering. However this property has little been used in biology, due to the lack of imaging schemes with temporal resolution compatible with sample deformation times.
We present the development of a fast P-THG microscope where excitation polarization is switched within microseconds between line scans using an electro-optic modulator to provide P-THG images free of artifact with millisecond temporal resolution. We also developed a Fourier transform-based analysis method for retrieving materials anisotropic properties from P-THG data.
We demonstrate several applications enabled by this approach. First, using a rotating linear polarization configuration, we use fast P-THG imaging to reveal molecular order changes in multilamellar lipid vesicles undergoing phase-transition upon heating. Second, we probe in vivo the anisotropic nature of endogenous microparticles flowing in the zebrafish embryo's inner ear (see Figure). Finally, we present a methodology to detect birefringence at micron scale in biological samples. This last application is based on the fact that no THG is obtained from isotropic samples excited with a circular excitation polarization whereas THG may be obtained from anisotropic samples in this condition. Overall, these developments establish a novel contrast modality for nonlinear biomicroscopy.

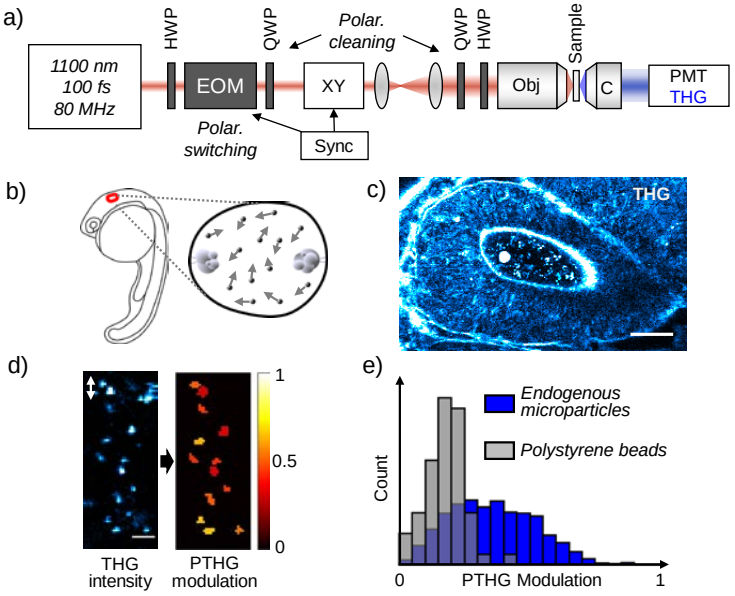

Figure: (a) Experimental set-up. (b-e) In vivo detection of anisotropy of microparticles in the zebrafish inner ear. (a) Schematic of the otolith cavity in a 2-days embryo. (b) THG imaging of the cavity. Scale bar 20 µm. (c) Extraction of the microparticles mean P-THG modulation. (d) Histogram of THG modulation for endogenous microparticles (blue) and injected polystyrene beads (grey) showing different polarization response. Adapted from [1].

[1] J. Morizet, G. Ducourthial, W. Supatto, A. Boutillon, R. Legouis, M.-C. Schanne-Klein, C. Stringari, E. Beaurepaire, "High-speed polarization-resolved third-harmonic microscopy," Optica (2019).