

FAST P-THG MICROSCOPY FOR BIOMATERIALS CHARACTERIZATION

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Polarization-resolved third-harmonic generation (P-THG) is a sensitive probe of materials anisotropy and molecular ordering. However this property has little been exploited used so far in biological tissues, due to the lack of imaging schemes with suitable temporal resolution.

We present here 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 have also developed a Fourier transform-based analysis method for retrieving materials anisotropic properties from P-THG data.

We demonstrate several novel applications enabled by this approach. First, using a rotating linear polarization configuration, we used fast P-THG imaging to reveal molecular order changes in multilamellar lipid vesicles undergoing phase-transition upon heating. Second, we probed in vivo the anisotropic nature of endogenous microparticles flowing in the zebrafish embryo's inner ear (see Figure). Finally, we developed a methodology to detect birefringence at micron scale in biological samples such as adult *c. elegans* worms. This last application is based on the fact that no THG is obtained from isotropic samples excited with a circular excitation polarization whereas THG possible in anisotropic samples. Overall, these developments establish a novel contrast modality for nonlinear biomicroscopy and show that P-THG is well adapted for characterizing materials anisotropy in biological environments.

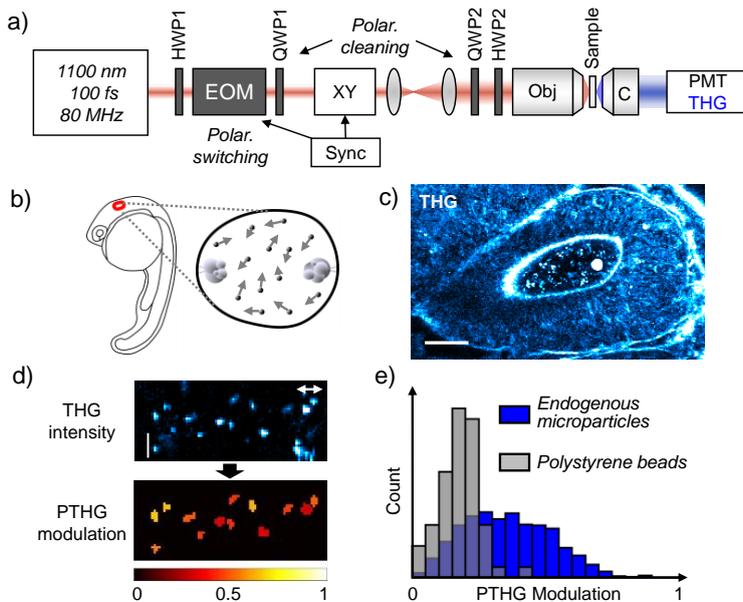


Figure: (a) Experimental set-up. (b-e) In vivo detection of anisotropy of microparticles in the zebrafish inner ear. (a) Schematic of the otolith cavity in a 2-days embryo. (b) THG imaging of the cavity. Scale bar 20 μm. (c) Extraction of the microparticles mean P-THG modulation. (d) Histogram of THG 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. Beaufepaire, "High-speed polarization-resolved third-harmonic microscopy," (submitted).