Polarized third-harmonic generation microscopy for the characterization of myelin and blood in brain tissue

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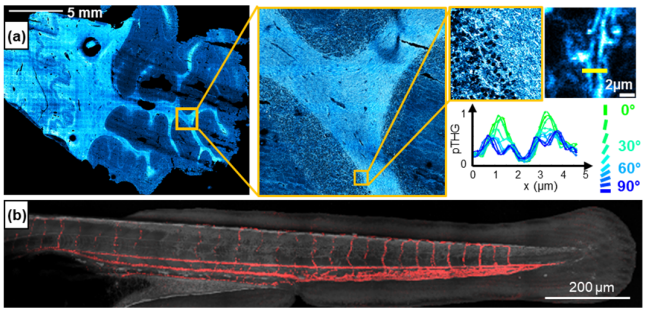
Multiphoton microscopy is widely used for 3D imaging of biological tissue. Third harmonic generation (THG) is a multiphoton contrast modality that highlights optical heterogeneity and interfaces. This results in morphological images of unstained tissues. THG signals can be efficiently produced using 1 MHz femtosecond infrared sources, which were recently developed for deep-tissue three-photon (3P) microscopy. [1] Thus, THG microscopy is currently gaining interest for tissue imaging when used in conjunction with 3P fluorescence. In recent years, our group has explored the dependence of biological third-harmonic generation (THG) signals on incident polarization and wavelength theoretically and experimentally.[2-7] In particular, we have performed a systematic characterization of polarized THG (pTHG) signals from individual axons in various systems, including live zebrafish spinal cords and fixed mouse and human brain tissues.[2] Myelinated axons are visible in THG images because of the high refractive index of myelin. We found that axons exhibit complex pTHG profiles that depend on axon morphology (axon diameter, myelin thickness) that cannot be elucidated by traditional numerical models of nonlinear microscopy. Aberrations caused by vertical micro-interfaces (e.g., water/lipid) appear to be a significant contrast mechanism in polarized THG imaging. We have developed appropriate numerical strategies to account for this effect. [2,3] This approach opens the way to quantitative analyses of pTHG images and myelin mapping at different scales. On the experimental side, we introduced experimental strategies for efficient polarized THG imaging, based either on line-by-line polarization switching using an electro optic modulator [4] or pulse-to-pulse polarization multiplexing combined with time-correlated photon counting.[5] We introduced a Fourier-based strategy for analyzing pTHG data and demonstrated that this approach can detect birefringence [4] and be useful for characterizing biominerals, such as kidney stones. [6] Additionally, we recently demonstrated that THG with excitation in the 1200–1300 nm range exhibits strong three-photon resonance enhancement due to hemoglobin's Soret absorption band. This wavelength-dependent response can be used to selectively probe red blood cells in THG images.[7] Overall, these results establish that multiparametric THG offers novel contrast modalities of interest for biomedical research.



Multiparametric THG imaging of biological tissues.
(a) Multiscale **polarized THG** imaging of an *ex vivo* human brain sample. THG highlights **myelin-rich areas** and pTHG provides structural information at the axon scale. *See Morizet, Optica 2025.*
(b) **Multi-wavelength THG** imaging of a live zebrafish embryo. Resonant THG in hemoglobin highlights **red blood cells**. *See Ferrer Ortas, LSA 2023.*

[1] Guesmi et al, Light Sci Appl (2018) https://doi.org/10.1038/s41377-018-0012-2
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[3] Morizet et al, Optica (2021) https://doi.org/10.1364/OPTICA.421257
[4] Morizet et al, Optica (2019) https://doi.org/10.1364/OPTICA.6.000385
[5] Gleeson et al, ACS Phot (2024) https://doi.org/10.1021/acsphotonics.4c00660
[6] Gleeson et al, ACS Phot (2023) https://doi.org/10.1021/acsphotonics.3c00651
[7] Ferrer Ortas et al, Light Sci Appl (2023) https://doi.org/10.1038/s41377-022-01064-4