

THIRD-HARMONIC GENERATION MICROSCOPY: PHASE-MATCHING, EPIDETECTION, AND EMBRYO IMAGING

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Signal level in coherent nonlinear microscopy is defined by the coherent interplay between sample structure and field distribution near focus. We analyze signal buildup in THG microscopy as a function of sample shape and focal field distribution. We show that focal field engineering using non-standard beams can be used to alter phase-matching conditions and can provide sub-micrometer information about sample structure [1]. We also address the issue of epidetecting forward-emitted light backscattered in a turbid medium. Theory and experiments indicate that in general, no significant TH signal can be epidetected from a biological structure embedded in a transparent medium, as opposed to direct backward emission from e.g. metal nanoparticles. In the case of a biological structure embedded in a turbid medium, epidetection is possible when harmonic light can exit the tissue before being reabsorbed, and epicollection critically depends on the microscope field-of-view because backscattered light is essentially diffusive [2]. This analysis provides guidelines for optimizing epidetection in THG, SHG, or CARS imaging of thick tissues.

Finally, we extend our previous studies on embryo imaging [3] and show that THG microscopy can provide a 3D-resolved dynamic description of morphogenesis while being fully compatible with other femtosecond techniques.

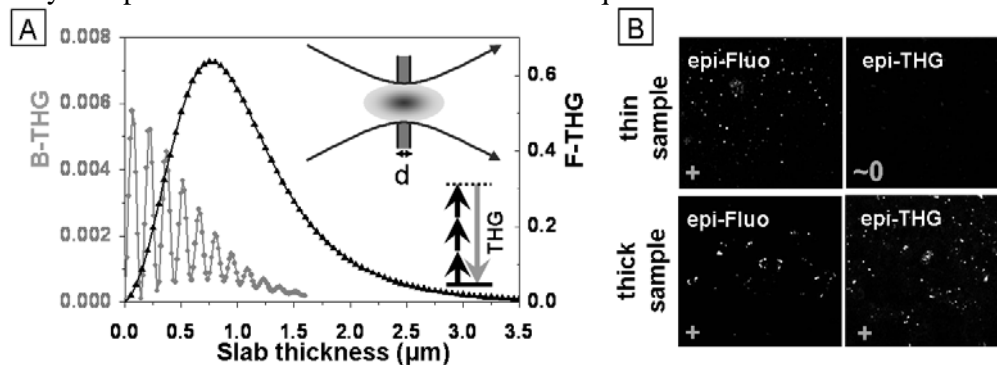


Fig 1. (A) Forward and backward THG from a sizeable dielectric structure in a transparent medium. (B) Epidetection of two-photon and THG signals in scattering tissue (adapt from 2).

[1] Olivier & Beaurepaire, Opt. Express 16, 14703 (2008).

[2] Débarre, Olivier & Beaurepaire, Opt. Express 15, 8913 (2007).

[3] Débarre et al, Opt. Lett. 29, 2881 (2004); Supatto et al, PNAS 102, 1047 (2005).