THIRD HARMONIC GENERATION MICROSCOPY OF COLLAGEN IN HEMATOXYLIN AND EOSIN STAINED BIOLOGICAL TISSUES

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KEY WORDS: Nonlinear laser scanning microscopy, second harmonic generation microscopy, third harmonic generation microscopy, histopathology, tissue collagen.

Multimodal nonlinear laser scanning microscopy is a powerful technique for noninvasive biological imaging. A few nonlinear signals can be generated simultaneously at the focus of a microscope objective, including second-harmonic generation (SHG) and third-harmonic generation (THG). Collagen, the main constituent of extracellular matrix in the biological tissue can be visualized with SHG microscopy, and can be used for cancer diagnostics [1]. Changes in the collagen structure are observed in hematoxylin and eosin (H&E) stained histology sections, which adequately contrast cell nuclei from the cytoplasm and extracellular structures and are referred to as the gold standard for histological investigations [2].

Figure 1: SHG image of collagen in H&E stained colon tissue (left) and THG image (logarithmic scale) from the same sample area (right). Size 350 × 390 µm.

Although collagen fibers are visualized with SHG microscopy, in this work, THG microscopy is also used to characterize the collagen and obtain additional structural information for cancer diagnostics. In addition to collagen, THG also reveals cell nuclei and enables to investigate the interaction between the cells and surrounding extracellular matrix in the cancerous tissue.