

# QUANTITATIVE ANALYSIS OF EPI-COLLECTED SECOND HARMONIC GENERATION IN BIOLOGICAL TISSUES

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As a novel modality of optical microscopy, second harmonic generation (SHG) provides attractive features including intrinsic optical sectioning, noninvasiveness, and high-penetrability. To apply SHG imaging in biomedical application, epi-collection of backward propagating SHG is necessary. Due to the phase matching constraint in nonlinear optical theory, SHG from thick tissues prefers forward propagation. SHG propagating in backward direction may originate from direct backward generation and backscattering of forward SHG. Here we provide a quantitative analysis to discriminate the contributions of backward generation and backscattering to the epi-collected SHG in mouse dermis and skeletal muscle tissues. We found that in both tissue types, forward SHG of similar intensities were observed. However, backward SHG from muscle fibers was much weaker than backward SHG from collagen fibers in dermis. With the aid of a frequency doubled laser, backscattering efficiencies in thick muscle and dermis tissues at SHG wavelength were estimated to be 1% and 4%, respectively. Compared with epi-collected SHG intensities in these tissues, backward-generated SHG power was found to be diminishing in muscle fibers, but backward-generated SHG power can reach 15% or more of forward SHG power in collagen-based tissues. Therefore, backward SHG is dominated by backscattering of forward SHG in muscle tissues. For collagen-based tissues such as dermis, on the other hand, the directly backward-generated SHG from the asymmetric bipolar emission profile is the dominant source for epi-collected SHG. Therefore, based on our results, epi-SHG microscopy would be more feasible with collagen-based tissues than with muscle fibers.