

# SIGNAL IMPROVEMENT IN MULTI-PHOTON MICROSCOPY BY REFLECTION WITH SIMPLE MIRRORS NEAR THE SAMPLE

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In multi-photon-induced fluorescence and multi-photon-induced higher harmonic generation, restriction of signal generation to a single focal point permits that all emitted photons can contribute to image formation if collected, regardless of their path through the specimen. Often the intensity of emitted light is rather low in biological specimens. We developed a method to significantly increase the fraction of photons collected by an epi (backward) detector by placing a simple mirror, an aluminum coated coverslip, under the sample. Samples investigated include fluorescent test slides, collagen gels, starch granules in potato slices, and intact mouse skeletal muscles. Quantitative analysis revealed an intensity increase of second and third harmonic generated signal in skeletal muscle of nine- and seven-fold respectively, and of fluorescent signal in test slides of up to two-fold. Our approach thus allows significant signal improvement in situations where a forward detection is impossible, e.g. due to the anatomy of animals in intra-vital microscopy.

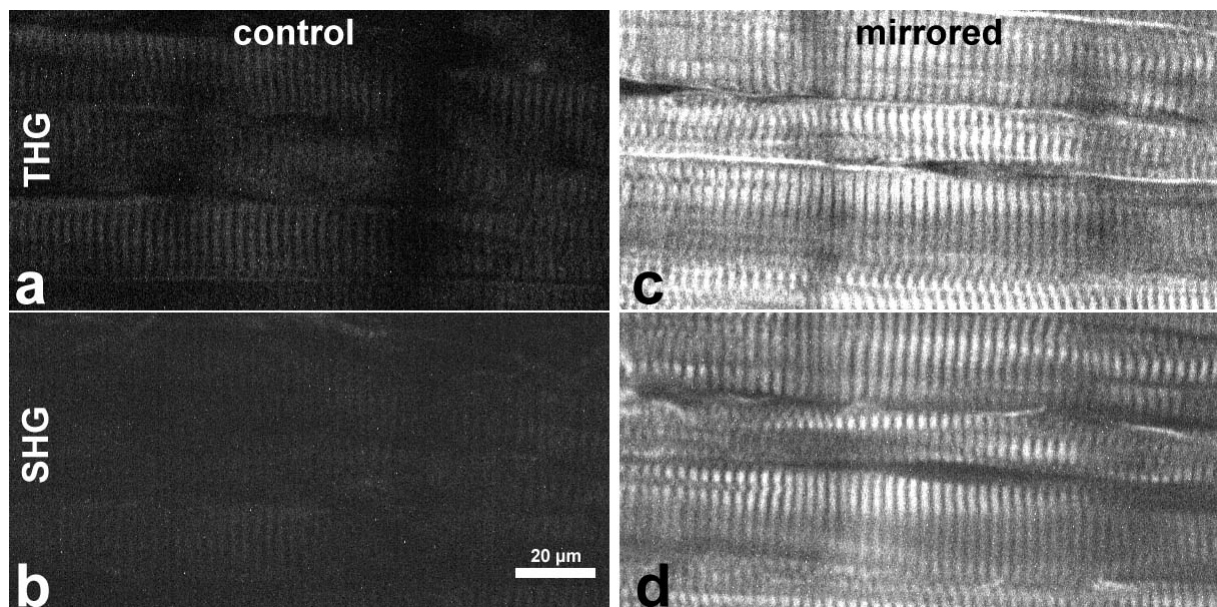


Figure: Higher harmonic imaging in the mouse cremaster muscle. Illumination with 1270 nm. THG (a,c) and corresponding SHG (b,d) images recorded with backward detectors. At identical recording and image processing parameters, intensity was very low without mirror (a,b) while mirrored images (c,d) are already partially saturated. Note vertical shadows due to structures in other focal planes.