

Functional imaging of biological samples by Second Harmonic Generation

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Recently, Second Harmonic Generation (SHG) has been applied to an increasing number of applications in imaging of biological samples, using both extrinsic and intrinsic SHG [1, 2]. Chiral dyes have been employed for the measurement of membrane potential in artificial vesicles and in live cells. With its most recent advances, SHG promises to become an essential tool for the investigation of electrical activity in cells and live tissues. Intrinsic SHG, on the other hand, has been revealed in a number of highly ordered biological samples, including connective tissue and muscle tissue. We have developed an SHG microscope for the functional imaging of cells and tissues. Imaging of different types of cells stained with RH-237 dye (Fig. 1A-D) has revealed that the sensitivity of SHG to transmembrane potential differs between normal and tumor cells [3], possibly due to the different physical and chemical properties of the plasma membrane. Our most recent work is aimed at optimizing

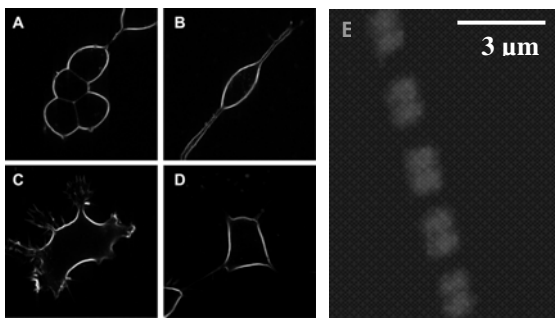


Figure 1. A-D: SHG images of live cells stained with RH 237. (A: HEK293; B: SY5Y; C: Balb/c3T3; D: SV3T3). The images dimensions are 50x50μm. **E:** SHG image of a muscle myofibril.

imaging conditions for real-time observation of electrical activity in cultured neurons and tissue slices. The apparatus has also been applied to imaging of muscle tissue (Fig. 1E). The strong dependence of the SHG signal on the presence of the highly ordered array of thick myosin filaments in the sarcomeres renders SHG a very interesting tool for the diagnosis of muscle pathologies leading to a loss in the order of sarcomeric structures. Also, because the most likely primary source of SHG is the molecular motor myosin, this technique may allow the measurement of structural dynamics of myosin in its native biological context. A comparison between fibers and myofibrils revealed a strong limitation in spatial resolution of SHG imaging in the fibers, whereas sub-sarcomeric structures could be well resolved within a myofibril. The polarization-dependence of the SHG signal combined with a simple model allows the measurement of a characteristic angular parameter describing the orientation of the SHG emitters within the symmetries of the muscle system.

[1] P.J. Campagnola and L. Loew. “Second-harmonic imaging microscopy for visualizing biomolecular arrays in cells, tissues and organisms” *Nat. Biotechnol.* **21**:1356, 2003.

[2] W.R. Zipfel, R.M. Williams and W.W. Webb. “Nonlinear magic: multiphoton microscopy in the biosciences” *Nat. Biotechnol.* **21**:1369, 2003.

[3] L. Sacconi, M. D'Amico, F. Vanzi, T. Biagiotti, R. Antolini, M. Olivotto and F.S. Pavone. “Second-harmonic generation sensitivity to transmembrane potential in normal and tumor cells” *J. Biomed. Opt.* **10**(2):024014, 2005.