Interferometric – Second Harmonic Generation Microscopy

Over the last decade, Second Harmonic Generation (SHG) microscopy has emerged as an effective imaging tool in biology. Like in two-photon excited fluorescence (2P), this type of laser scanning microscopy is characterized by an intrinsic 3D sub-micron resolution robust upon light scattering which allows for higher image depth when compared to confocal microscopy. SHG is a nonlinear optical process in which highly polarizable and non-centrosymmetric structures emit photon at exactly half the excitation wavelength. The emitted light results from the coherent sum of the electromagnetic fields generated by every single SHG emitters and thus scale quadratically with the number of aligned molecules (sharing the same polarity). Indeed, adjacent molecules of the same polarity will emit strong SHG signal due to constructive interference while the SHG signal will vanish in the case of adjacent molecule of opposite polarity.

In SHG microscopy, because the measured signal is proportional to $|\chi^{(2)}|^2$, the measured signal is insensitive to the orientation of the polarity. Over the recent years, we have developed interferometric SHG (I-SHG) to gain information about the level of orientation in biological samples. Originally developed to characterize non-centrosymmetric crystals [1,2], I-SHG is based on the measurement of the phase of the SHG signal. In the past years, its potential for tissue imaging has been demonstrated in different proteins, such as myosin from skeletal muscle [3] (see figure 1) and collagen from tendon and cartilage [4,5]. Having recently solved one of the main drawback of I-SHG [6], namely the long imaging time, we have recently demonstrated the possibility of using I-SHG to record the dynamical evolution of microtubule polarity in mitotic spindle from zebrafish embryos (in preparation).

Figure 1: Left - SHG image of skeletal muscle. Right – I-SHG image of skeletal muscle. With I-SHG, the bands are composed of two structures with opposite polarity.