# SHG and THG made easy with spectrally tunable non-descanned detection

**Susanne Holzmeister, Leica Microsystems CMS GmbH, Mannheim, Germany**

The term multiphoton microscopy is mostly used when referring to two-photon excitation (2PE). The advantages are widely known: The red-shifted IR laser lines cause less photodamage and penetrate deeper into the tissue. At the same time the focus is inherently confined in z - no pinhole for sectioning is needed and the fluorescence can be collected close to the sample and with high collection efficiency, in a so-called non-descanned position. These characteristics made 2PE a tool widely used in deep imaging of biological tissue such as brain and other organs in live or fixed specimen. It is important to note that the advantages also apply and are even enhanced for higher order excitation processes: Second harmonic generation (SHG) and third harmonic generation (THG).

What is interesting about these physical processes, why are they useful tools in biological research? Various diseases are related to structural changes of collagen fibers, myosin, the blood system... Labeling these structures or organelles is possible but mostly time-consuming and challenged to change the original behavior. Therefore, SHG has emerged as powerful, label-free imaging modality to visualize fibrillar collagen in diverse tissues, and THG is used to visualize blood flow or refractive index mismatches at nerve fibers.

SHG signals occur from large non-centrosymmetric structures with a periodic alignment. These structures can double the frequency of the infrared pulsed excitation light. In conclusion, no labels need to be incorporated and since the light is not absorbed but scattered, photodamage does nearly not occur.

In analogy to SHG, THG occurs at exactly one third of the incoming pulsed laser light wavelength. Using laser sources with wavelengths up to 1300 nm, THG can be detected in the visible at 430 nm. Since THG occurs at locations of refractive index mismatch, it provides information content related to that of phase contrast microscopy but due to local confinement of the focal spot, the phase contrast information is even obtained in three dimensions.

Spectrally tunable non-descanned detection in combination with pulsed excitation sources with an extended tuning range up to 1300 nm enables to select a specific narrow detection bandwidth at exactly double or triple the frequency **of any excitation wavelength** to visualize unlabeled structures like collagen, myosin, microtubules or hemoglobin in addition to or instead of conventional fluorescent signals. Without the need of a dedicated filter, e.g. live blood flow tracking becomes feasible. In the event of resonance enhancement, absorption of the THG excitation light leads to an enhancement of the signal intensity – this happens e.g. with hemoglobin and enables blood flow tracking.

As consequence, spectral detection at the non-descanned position adds important functional information to laser scanning microscopy experiments.

In conclusion, tunable spectral non-descanned detection enables easy realization of label-free multiphoton experiments and provides novel insights into biology –without the need of time-consuming fluorescence labeling!