**Deeper and faster: new tools for nonlinear bioimaging**

L. Bonacina

*Department of Applied Physics*

*University of Geneva, Switzerland*

e-mail: luigi.bonacina@unige.ch

While the majority of studies on the nonlinear properties of micro- and nano-structures focus on the production of distinct signals, like second and third harmonic, we recently showed that the new generation of rugged commercial dual output sources designed for microscopy (OPO, OPA) can easily expand the accessible parameter space and enable the simultaneous excitation and detection of multiple nonlinear emissions from individual objects, including several harmonics and parametric signals derived from different frequency mixing processes.1 This rich response, which in our case features 10 distinct emissions and spans from the deep ultraviolet to the short-wave infrared, is demonstrated using various metal-oxide materials (harmonic nanoparticles, HNPs) while being characterized and numerically simulated both in the temporal and spectral domains.2 HNPs offer excitation flexibility and quasi-instantaneous optical response and their use as imaging probes can be leveraged to increase speed in scanning microscopy, achieving dwell times as short as 1 nanosecond, faster than typical fluorophore lifetimes.

Our strategy combining nonlinear optical excitation and HNPs labelling is illustrated in the context of the preclinical assessment of cell therapies. Specifically, we apply it to a recently proposed protocol based on the use of mesenchymal stem cells to regenerate dystrophic muscles affected by Duchenne myopathy. Our imaging experiments reveal HNP detection in tissues at depths greater than 1 mm,3 as well as high-sensitivity detection of labelled stem cells in whole blood and characterization of their morphology and aggregation-state at framerates exceeding 15'000 images per second.4 At these imaging rates, blood volumes consistent with preclinical animal research could be monitored in a matter of minutes without the need of sample prefiltration.

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