TAILORED BROADBAND PULSES FOR MULTIMODAL NONLINEAR IMAGING OF BIOLOGICAL SAMPLES

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Multimodal nonlinear optical microscopy relies on the use of complementary contrast mechanism based on intrinsic structural and chemical properties of the sample. Since nonlinear signal generation happens mainly in the focal volume, this method is ideally suited for 3D imaging of tissues. The label-free, non-invasive technique encounters a rapidly growing interest thanks to multiphoton imaging platforms becoming more accessible and to progress in laser technology. Indeed, the availability of pulsed, ultrashort lasers has been a key development to achieve high signal levels at moderate mean power.

In our setup, a pulse shaper allows adapting the pulse spectral and temporal profile for optimal imaging. The shaper permits application of complex spectral phase and amplitude masks, as well as being convenient for suppression of the dispersion affecting the 10fs pulses after travel through microscope objectives. While pulses compressed back to the Fourier limit produce the strongest signal for all nonlinear effects, they are also the most prone to induce photodamage. We show how introducing phase distortions allows controlling the ratio between different nonlinear effects. Despite the use of a single laser, spectra over the whole relevant range for biological imaging up to 3000cm\(^{-1}\) can be accessed thanks to the broad bandwidth of the pulses[1]. Alternatively, the available excitation energy can efficiently be concentrated into a single Raman transition.

The capabilities of the setup are demonstrated using the most commonly used nonlinear effects: Second Harmonic Generation (SHG), Two-Photon Fluorescence (TPEF) and CARS (Coherent Anti-Stokes Raman Scattering). Imaging is performed on tissues relevant for multimodal imaging and its applications in medicine: skin and spinal cord. The use of this technique for the yet little explored imaging of plant cells is illustrated with images of leaves of the moss *Plagiomnium rostratum*.