EXPLORING SATURATION PROCESSES IN NEAR-IR TRANSIENT ABSORPTION MICROSCOPY

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In the last decades, non-linear optical processes have captured the attention of life scientists for the development of new super-resolved microscopy techniques. Non-linear optical microscopy goes hand-in-hand with the exploitation of the near-infrared (near-IR) part of the spectrum. It was mainly introduced to overcome the scattering problem in fluorescence imaging of thick samples, resulting in an increase in imaging depth, in a greater molecular specificity, and in an enhancement of contrast and resolution [1].

In order to broaden the range of available targets and provide novel contrast mechanisms in weakly or non-fluorescent samples, absorption-based techniques coming from optical spectroscopy were intensely studied and coupled with scanning microscopy. This opens the possibility to explore saturation and differential techniques for the circumvention of the diffraction limit also in non-fluorescence-based methods [2,3,4].

In our pump-probe (or transient absorption) microscope, two femtosecond pulsed laser beams are coupled with a commercial upright Nikon microscope. They are generated by an OPO (Optical Parametric Oscillator), which is pumped by a mode-locked Ti:sapphire laser, and they are used to investigate ultrafast (sub-picosecond) dynamic properties of the sample with high spatial and temporal resolution, and high sensitivity. The absorption variations of the probe beam, in presence and in absence of the pump beam, are detected. In order to identify this weak signal, which is buried into the background, a fast modulation of the pump beam is added, thus, only the signal of interest will be translated into a modulation of the probe, which is detected in transmission and filtered by a lock-in amplifier. Moreover, the superimposition of a third beam allows to explore super-resolution capabilities, taking advantage of spatially controlled absorption or stimulated emission saturation effects. All the wavelengths can be tuned in the near-IR region and the time delay between the pulses can be modified through a translation stage in order to study different transient absorption mechanisms and their dynamics in biological or nanomaterial samples.

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