

WHITE LIGHT LASER (WLL) SPECTRAL CONFOCAL IMAGING.

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The development of several new fluorescent molecules and the need for more accurate fluorescence signatures including lifetime fingerprint indicate the necessity of more flexible light sources than the ones normally used in confocal microscopy¹. In general, one of the main limitations in confocal microscopy is due to the fact that experimental design is often hampered by the limited choice of excitation wavelengths. In the last years, also in conjunction with those technical advances related to the improvement of multiphoton microscopy, a new generation of laser sources has been proposed and used, mainly based on supercontinuum laser technology². Such white light laser (WLL) sources constitute the perfect combination with modern spectral confocal microscopes. In particular, we recently tested the performances of a Koheras SuperK compact WLL coupled to the Leica TCS SP5 AOBS system endowed with fast resonance scanners and complemented with a Becker-Hickl lifetime system. Such a combination has been used for multiple fluorescence imaging, up to 8 fluorophores discriminated, for lifetime measurements using the 90 MHz repetition frequency in the visible excitation range and, as prosecution of earlier experiments based on a home-made system³, the WLL has been used for FRET measurements using the acceptor photobleaching and spectral approaches. In all these cases we benefited of the flexibility of the excitation wavelength choice that results in a uncommensurable advantage when coupled to an efficient spectral device like the AOBS technology mounted within the confocal scanning head. Experiments have been carried out using polyelectrolyte based fluorescent objects and different cellular populations.

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