

WHITE LIGHT LASER (WLL) IMPROVE SPECTRAL CONFOCAL IMAGING AND FRET

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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 wavelengths¹. In the last years, 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. They allow the application of any new fluorescent molecules and they guarantee more accurate fluorescence signatures including lifetime fingerprint.

In particular, we recently tested the performances of a Koheras SuperK compact WLL coupled to the Leica TCS SP5AOBS 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 (Forster Resonance Energy Transfer) measurements using the acceptor photobleaching and spectral approaches. In particular in FRET imaging a quantitative data interpretation can be difficult due to donor-acceptor spectral overlap which leads to contaminations of the FRET signal. These contaminations are worsen by the limited choice of excitation wavelengths available on conventional microscopes. We benefited of the flexibility of the WLL excitation wavelength choice as well as the capability of performing excitation spectra directly on the sample under investigation: on one hand it has been possible to fully characterize the FRET couple; on the other it has been possible to optimize the excitation wavelengths thus limiting the spectral contaminations. Experiments have been carried out using polyelectrolyte based fluorescent objects and different cellular populations.

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