HIGH SENSITIVITY NDD FLIM
AND PATTERN MATCHING BASED FLUOROPHORE IDENTIFICATION

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Confocal microscopes with multiphoton excitation and non-descanned detection (NDD) can penetrate deep into tissue. The combination with Fluorescence Lifetime Imaging (FLIM) extends the capability of such an instrument for concentration mapping of specific molecules, superior discrimination against autofluorescence as well as time resolved FRET (FLIM FRET).

For optimal detection efficiency the fluorescence light is collected directly above the objective and guided to a PMA Hybrid detector combining GaAsP cathode sensitivity with excellent timing performance. The NDD based FLIM adapter replaces the standard objective holder and is easy to mount. A modular detection assembly allows to perform both confocal and NDD FLIM measurements.

The fluorescence lifetime of fluorophores is strongly dependent on their photophysical properties and local environment. Together with the absorption and emission profile detected in different spectral channels, the fluorescence decay can act as a fingerprint for a dye in a specific environment. We present a Pattern Matching analysis technique that allows to identify selected patterns consisting of fluorescence decays in different spectral excitation and detection channels. The technique is easy to apply and allows for an excellent distinction of different fluorophores, changes of fluorescence parameters due to differences in the environment as well as their discrimination from autofluorescence in biological samples.

We show that five different stainings with similar absorption spectra can be separated allowing for multi-label detection inside biological cells. The specificity of the Pattern Matching analysis is sufficient to even image simultaneously Golgi apparatus, actin and nucleus all stained by the same dye ATTO 488.