

# FEMTOSECOND NONLINEAR MICROSCOPY AND PHOTOMANIPULATION BASED ON A BROADBAND TUNABLE ER/YB:FIBER SYSTEM

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State-of-the-art scanning microscopes employ up to four gas and solid-state lasers for linear excitation of different fluorophores. To date, commercial multi-photon systems include costly Ti:sapphire femtosecond oscillators. Clearly, there exists a strong demand for versatile, inexpensive and turn-key sources for linear and nonlinear excitation in bioimaging. Here, we present such a setup based on a single mode-locked Er: fiber laser. This system provides ultrashort pulses for nonlinear microscopy at 1550 nm (fundamental), from 1050 nm to 1400 nm (tailored supercontinuum) and at 775 nm (second harmonic). The low-noise supercontinuum is generated in a germanosilicate single-mode fiber. This technique offers robust frequency conversion in the near infrared with minimum acoustic pickup. Therefore, highly stable visible pulses tunable from 480 nm to 700 nm are available via frequency doubling. This feature allows us to precisely excite fluorophores absorbing in the green to near-infrared region. Due to the high peak power of the femtosecond pulse trains, also multiphoton techniques are accessible, including targeted photomanipulations. In order to characterize the system, we have measured fringe-resolved autocorrelation traces in the confocal plane via two-photon absorption in semiconductor photodiodes with adequate bandgap. Pulse durations after transmission through the entire laser scanning microscope of 150 fs at 1.55  $\mu\text{m}$  and 200 fs at 780 nm are achieved without precompensation. Employing a SF11 prism compressor results in pulse widths as short as 33 fs. The pulse length is below 60 fs in the entire tuning range between 1050 nm and 1400 nm. Owing to the right choice of prism material, a compressor with a tip to tip distance of 37 cm is appropriate to compensate for second- and third-order dispersion introduced through the scanning optics and the objective lens. In order to boost the average output power for highly nonlinear applications up to 550 mW, the setup was extended by a compact Yb: fiber amplifier pumped by two single-mode diodes at 976 nm. The Yb: amplifier is seeded with the broadband supercontinuum tuned to a center wavelength of 1050 nm. The amplifier output spectra are centered at 1050 nm and have a bandwidth up to 50 nm (FWHM). Pulse compression with a pair of gratings (600 lines per mm) provides an in-focus pulse length of 77 fs. With sub-100 fs performance and an average power of more than 500 mW, the compact Yb-extension offers output parameters comparable to typical Ti:sapphire systems. As an example for 3-dimensionally confined photomanipulations we induced DNA damage via multiphoton absorption. Collateral damage in other parts of the cell is minimized because three-photon absorption occurs only in the confocal volume. Previously, these experiments were performed with a Ti:sapphire femtosecond laser operating around 800 nm. In this wavelength regime, a mixture of different types of DNA-lesions is produced, including UV-photoproducts and DNA strand breaks. This situation hampers the study of individual DNA repair pathways. Here, we determine how the type of DNA damage triggered by three-photon absorption varies depending on the irradiation wavelength. To this end, we extended excitation to 1050 nm and characterized the spectrum of DNA damage using specific antibodies and GFP-tagged repair factors (e.g. XPC or XRCC1). Our comparative analysis suggests that multi-photon absorption at a wavelength beyond 1  $\mu\text{m}$  favors the generation of DNA strand breaks compared to UV photoproducts (6,4-PP and cyclobutane pyrimidine dimers).