

DISSECTING DNA REPAIR PATHWAYS BY QUANTITATIVE DNA MICROIRRADIATION WITH FEMTOSECOND LASER PULSES

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Laser-mediated DNA microirradiation is a powerful and popular method for the study of the spatiotemporal dynamics of DNA repair in live cells. In contrast to the commonly employed UV/VIS laser wavelengths, irradiation with NIR femtosecond laser pulses provides 3D confinement of the damaged region and reduced collateral damage. The exact nature of the DNA damage induced by these pulses as well as the underlying mechanisms of light-matter interaction are still poorly characterized. To enable a systematic and quantitative analysis of the response of DNA to femtosecond pulses in the context of the living cell we developed a high-efficiency, low-drift and multicolour femtosecond Er: fiber laser system and integrated it into a dual-scanner photomanipulation/confocal imaging setup. This system enabled microirradiation of cellular DNA at four different wavelengths evenly spaced on the energy scale between 1030 nm and 515 nm with highly comparable characteristics: the pulse duration is constant (80 fs measured at the sample), pulses are chirp-free with transform-limited shape, the pulse trains have constant repetition rate (40 MHz), and the beam has a very high quality ($M^2 = 1.1$) [1]. We then performed quantitative analysis of DNA damage and repair endpoints including immunocytochemistry and imaging of the recruitment of DNA repair factors. Taking advantage of the precise design of our pulse parameters we could combine these measurements with the theoretical modeling of femtosecond laser-induced nonlinear energy deposition [2]. Our results demonstrate a striking correspondence of the cellular damage response to the type of interaction predicted by the theoretical model. We also show how our approach can be used to distinguish the direct induction of DNA strand breaks by femtosecond pulses from secondary damage elicited in the course of the DNA repair process.

[1] M. F. Schmalz, I. Wieser, F. Schindler, C. Czada, A. Leitenstorfer, E. Ferrando-May "Highly Standardized Multicolor Femtosecond Fiber System for Selective Microphotomanipulation of Deoxyribonucleic Acid and Chromatin" *Opt. Lett.*, **43**, 2877-2880 (2018)

[2] X. Liang, Z. Zhang, A. Vogel "Multi-rate-equation Modeling of the Energy Spectrum of Laser-Induced Conduction Band Electrons in Water" *Opt. Exp.*, **27**, 4672-4693 (2019)