

DNA DAMAGES INDUCED BY DIFFERENT LASER MICROTOOLS

Paulius Grigaravicius, Alexander Rapp, Shamci Monajembashi and Karl Otto Greulich
Institute for Molecular Biotechnology Jena, Department of Single Cell and Single
Molecule Techniques, Beutenbergstr. 11, 07745 Jena, Germany
E-mail: paulius@imb-jena.de

KEY WORDS: Microbeam, Laser-microtools, DNA Damage, DNA repair

ABSTRACT:

Microdissection and micromanipulation of cells and subcellular structures using microbeams and optical tweezers has several advantages over micromechanical handling by micro tools [1]. Nevertheless, these systems are known to induce photodamage to exposed living objects. Thus, it is very important to investigate damages induced by laser radiation of different quality, especially with regard to DNA damages.

Former studies using human lymphoblasts and lymphocytes, which were micro-irradiated with different light sources such as a cw Nd:YAG laser, a cw tunable Ti:Sa Laser (750-1064nm, 60-240 mW, 10-50 TJ/m²), mercury lamp and pulsed dye laser at wavelengths from 308 to 640 nm have demonstrated dramatic wavelength dependency of the generated DNA damages [2,3]. These studies used the Comet-assay, a single cell based technique to measure DNA fragmentation electrophoretically. We found that in general, extremely higher energy densities are required to induce DNA damages at longer wavelengths.

In the recent work we additionally use immunohistochemical staining, such as the detection of γ -H2AX foci, a marker for individual DNA double strand breaks, to examine the biological effects of micro-irradiation. Additionally a new setup is build, which comprises of a tunable femtosecond Ti:Sa laser system (Tsunami, Spectra Physics) coupled into an Axiovert 135M microscope (Carl Zeiss) via the epifluorescence illumination path. This setup enables localized micro-irradiation in a broad part of the spectrum using the fundamental (750-1000 nm), frequency-doubled (370-500 nm) and frequency-tripled (250-330nm) irradiation within the same system.

[1] K. O. Greulich, Micromanipulation by Light in Biology and Medicine -The Laser Microbeam and Optical Tweezers (Birkhäuser, Basel, 1999).

[2] A. de With, and K.O. Greulich, "Wavelength dependence of laser-induced DNA damage in lymphocytes observed by single-cell gel electrophoresis" *J. Photochem. Photobiol. B*, **30**, 71-76 (1995).

[3] S. K. Mohanty, A. Rapp, S. Monajembashi, P.K. Gupta, and K.O. Greulich, "Comet assay measurements of DNA damage in cells by laser microbeams and trapping beams with wavelengths spanning a range of 308 nm to 1064 nm" *Radiat. Res.*, **157**, 378-385 (2002).