

# INTRACELLULAR MANIPULATION OF SINGLE CELLS USING ULTRASHORT LASER PULSES: ABLATION OF MITOCHONDRIA AND CYTOSKELETON

Anaclet Ngezahayo<sup>1</sup>, Judith Baumgart<sup>2</sup>, Sabine Przemeck<sup>4</sup>, Kai Küttemeyer<sup>2</sup>,  
Lydia Kruppe<sup>1</sup>, Frank Witte<sup>3</sup>, Willem Bintig<sup>1</sup>, Holger Lubatschowski<sup>2,4,\*</sup>,  
Alexander Heisterkamp<sup>2,3</sup>

<sup>1</sup>Institute of Biophysics, Leibniz University Hanover, Hanover, Germany;

<sup>2</sup>Laser Zentrum Hannover e.V., Hollerithallee 8, 30419 Hanover, Germany;

<sup>3</sup>CrossBIT, Hanover Medical School, Feodor-Lynen-Str. 31, 30625 Hannover;

<sup>4</sup>Rowiak GmbH, Garbsener Landstr. 10, 30419 Hanover, Germany

\*E-mail: hl@rowiak.de

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**OBJECTIVE:** We investigated the manipulation of mitochondria and other cell organelles in fixed as well as living single cells by optical means and its effect on mitochondrial apoptosis.

**METHOD:** Ultra-short laser pulses were focused by a high numerical aperture objective. The high intensities created within the focal volume enabled visualization and accurate manipulation of cells at subcellular level. The visualization of the microfilaments as well as the microtubules was achieved by either staining microfilaments with Alexa 488 ® conjugated phalloidin or labelling actin with FP-635 and tubulin with GFP. Mitochondria were identified with MitoTracker ® Red FM and induction of apoptosis was traced by immunofluorescent staining with Propidium iodide and Annexin V.

**RESULTS:** Disruption of single cytoskeletal filaments as well as mitochondria demonstrated the high precision necessary in order to manipulate cell organelles.

Ablation of a single mitochondrion with repetition rates of 4.0 MHz or 80 KHz did not induce cell death. Manipulation of about 6 or 8 mitochondria per cell at a repetition rate of 4.0 MHz caused cell membrane blebbing within minutes. Staining positive for Annexin V and negative for Propidium iodide indicated early apoptotic changes.

**CONCLUSION:** Intracellular manipulation by optical means offers the possibility to elucidate cellular processes e.g. apoptotic pathways, particular with regard to the mitochondrial pathway, as mitochondria are targets for a variety of apoptosis-inducing drugs in cancer therapy.