Intracellular Manipulation of Single Cells using Ultrashort Laser Pulses: Mitochondria and Cytoskeleton

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Introduction

Life science makes already use of ultrashort laser pulses (femtosecond pulses) by imaging cells with multiphoton microscopy or biological tissue with optical coherent tomography. Mainly fs-laser with wavelengths near the infrared (NIR)-range are used. Here we present a method employing an NIR-fs-laser to dissect and manipulate fixed and living cells by optical means at subcellular level.
Cell components such as actin filaments and mitochondria play an important role in a variety of vital cell events e.g. cell dynamics and apoptosis, respectively.

Objective

Investigation into fields of application for cellular surgery by optical means in fixed as well as living single cells with special emphasis on induction of apoptosis.

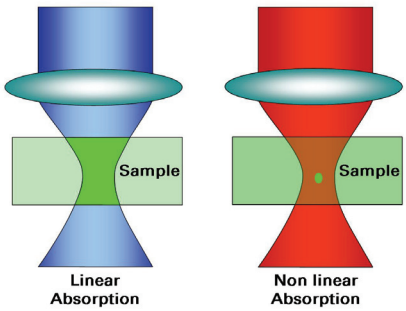
Methods

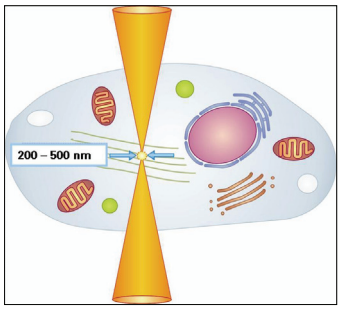
Cells

For disruption of cytosekeletal elements in fixed Chinese hamster ovary (CHO) cells and living Human osteosarcoma (U-2-OS) cells actin filaments were labelled with Alexa 488 ® conjugated phalloidin or expressed FP635-tagged actin, respectively. For ablation of single mitochondria in living bovine aortic endothelial (GM-7373) cells mitochondria were stained with MitoTracker Orange ®. For verification of apoptotic events following ablation of 6 to 8 mitochondria in living GM-7373 cells mitochondria were identified by MitoTracker Red FM ® and inductino of apoptosis was verified by Annexin V-FITC / propidium iodide staining.

Manipulations at subcellular level with Nanodissection System “CellSurgeon” by ROWIAK GmbH

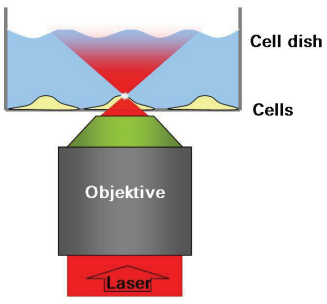
The Laser beam of a NIR-fs-laser was coupled into a commercially available microscope and tightly focused by a high numerical aperture objective. This resulted in very high intensities within the very small focal volume and induced non-linear processes (multiphoton absorption). As pulse duration was within the fs-range energy deposited amounted to only a few nanojoules per pulse. The combination of low energy deposited and a very small focal volume allowed for precise dissection and ablation of cellular components at subcellular level in single cells.

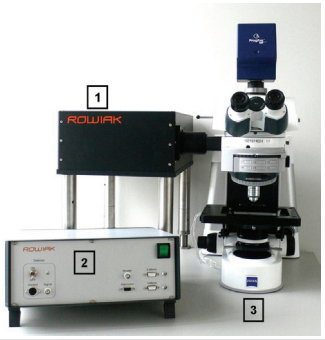




Left: Linear absorption – constant absorption coefficient (μ_a); non linear absorption - μ_a depends on intensity of light (fs–laser pulses) and enables a very small focal volume (< 1µm in diameter).

Right: The tightly focused fs-laser beam allows a high spatial selectivity and precise ablation without harming adjacent cells.



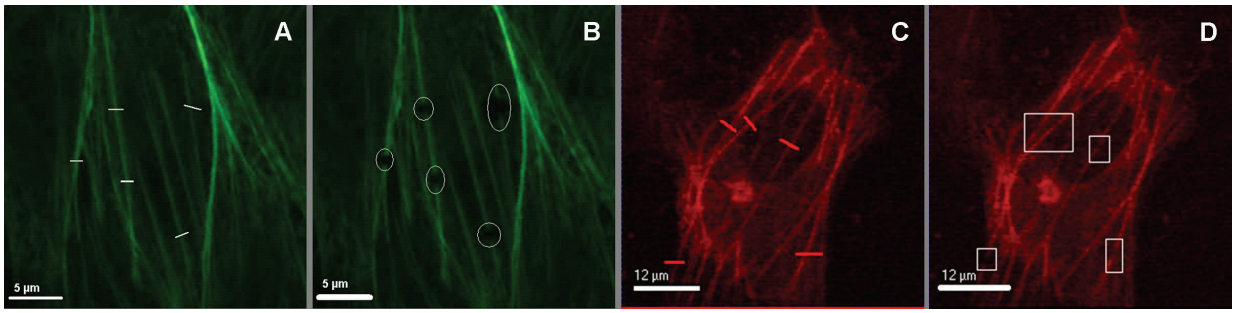


Left: Experimental set up for manipulation of adherent cells.

Right: Nanodissection system “CellSurgeon” by ROWIAK GmbH
1 transfer optics (couples laser beam into microscope)
2 electronic control unit
3 upright fluorescence microscope (Zeiss, Axio-Imager) (laser source not pictured)

Results

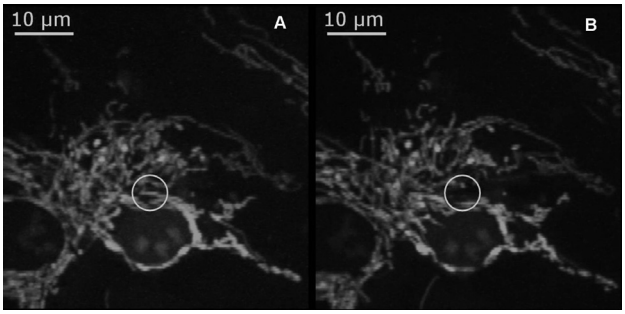
Disruption of cytoskeletal elements



Fixed CHO cells labelled with Alexa 488 ® conjugated phalloidin: A – before manipulation, B - after manipulation with pulse energy of 5.1 nJ at repetition rate of 80 MHz; manipulated areas highlighted

Living U-2-OS cells expressing FP635-tagged actin: C – before manipulation, D - after manipulation with pulse energy of 2.1 nJ at repetition rate of 80 MHz; manipulated areas highlighted

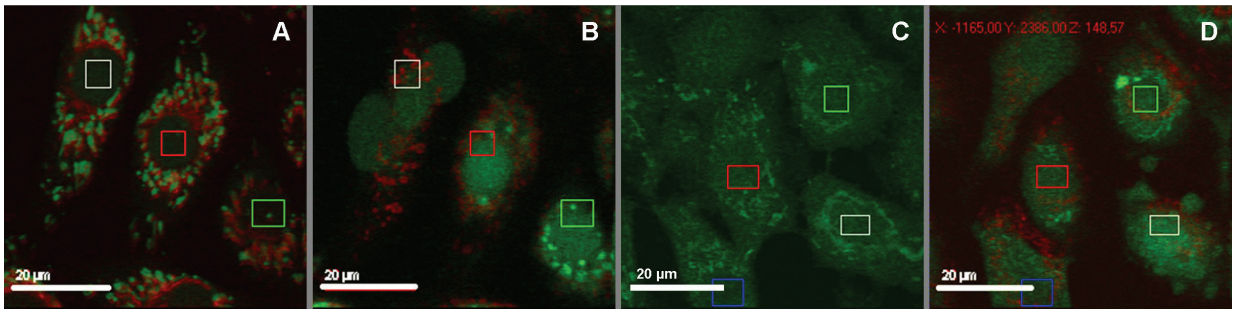
Ablation of single mitochondria



Living GM-7373 bovine aortic endothelial cells labelled with MitoTracker Orange ®
A - before manipulation
B - after ablation with pulse energy of 1 nJ at repetition rate of 80 MHz;
ablated mitochondria highlighted

(Heisterkamp *et al.* in *Methods in Cell Biology* 82: Chapter 9 (2007)

Induction of apoptosis



Living GM-7373 endothelial cells stained with Mito Tracker Red FM, H2DCFDA * ,Annexin V – FITC and Propidium iodide; manipulated cells highlighted with a white frame.
All cells were additionally stained with the green-fluorescent ROS-marker H2DCFDA as part of another experiment.

A - before ablation
B - 4.5 min after ablation of 6 mitochondria with pulse energy of 1.5 nJ at repetition rate of 4 MHz; membrane blebbing is clearly visible

C - before ablation
D - 2 min after ablation of 8 mitochondria with pulse energy of 1.5 nJ at repetition rate of 4 MHz; membrane blebbing is clearly visible

Conclusions

Optical cell surgery is a very accurate method and offers the possibility to investigate cellular processes. Here we have shown that it is feasible to selectively induce and monitor the mitochondrial pathway of apoptosis by ultrashort laser pulses in single cells. Thus enabling investigations into apoptosis without the need of chemical stimuli, what could be a valuable tool in cancer research.