Combined multiphoton imaging and cell surgery by femtosecond laser pulses

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Precise manipulation of single cell organelles by fs-nanosurgery offers many possibilities for the understanding of intracellular sequences and function of cell organelles. The imaging by multiphoton microscopy and the laser manipulation of living cells is a useful tool to handle the cells without damaging them.

1 Introduction

Medical treatments based on the cellular level, like cancer therapy, require the understanding of cellular processes and intracellular functions [1]. With the aid of a femtosecond laser it is possible to interrupt a sequence of events for example by ablating a predefined cell organelle. Therefore it is necessary to obtain a very precise image of the cell and its organelle. Multiphoton microscopy combines the possibility of three dimensional fluorescence imaging and manipulation of living biological tissue by using a femtosecond laser at high spatial resolution in the submicrometer range [2-6].

2 Methods

Imaging by femtosecond laser pulses is based on nonlinear absorption. When the laser beam is focused into a very small focal volume, the photon density rises high enough to induce multiphoton absorption within this volume [7]. Fluorochromes whose excitation maximum is in the UV or in the visible wavelength range are excited by two or three infrared photons. This nonlinear absorption occurs solely at the focal volume of the laser beam, thus limiting the induced fluorescence to this volume (fig. 1). Consequently there is no interfering fluorescence from the surrounding tissue which reduces otherwise the high spatial resolution. Additionally the use of infrared light implicates a high penetration depth into biological tissue of up to 200 micrometers due to the low absorption of the primary components of the cell like water, melanin and hemoglobin [9].

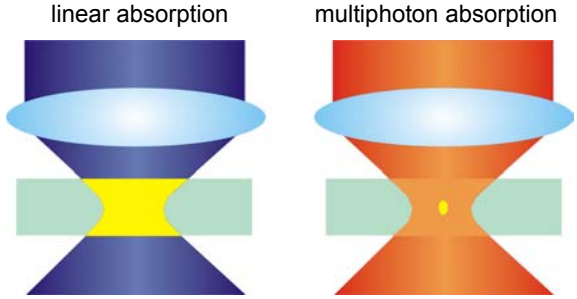

Fig. 1 *Linear absorption compared to multiphoton absorption. Due to the very narrow absorption volume in multiphoton absorption, photobleaching is reduced to the focal volume.*

For micromanipulation by femtosecond laser pulses, the pulse energy has to exceed the threshold for optical breakdown. At high pulse intensities, a plasma will be created by multiphoton ionisation of typically five or six photons. This plasma generates a very high concentration of free electrons and induces an outwardly propagating shock wave due to the local heating [10]. The occurring cavitation bubble causes disruption of the material. The use of high numerical objectives leads to a focal volume of a lateral extent of less than one micrometer, allowing the manipulation of single cell organelles which have typically the size of some micrometers. The dimension of the manipulation increases rapidly with increasing pulse energy. Thus, it is favorable to choose pulse energies as low as possible to realize very precise cuts.

Figure 2 shows the multiphoton microscopy image of the DAPI labeled rat1 fibroblast nucleus. The imaging was realized at a pulse energy of 0.1 nJ. Three cuts were realized inside the nucleus. At 0.54 nJ, the pulse energy is below the photobleaching threshold at approximately 0.73 nJ. Increasing the energy to a factor of ten times the imaging energy, which is about 20% exceeding the photobleaching threshold, leads to ablation of the material [12].

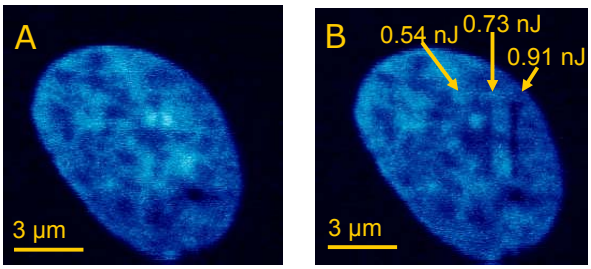

Fig. 2 *Fixed Rat1 fibroblast, the nucleus is DAPI labeled. (A) The nucleus before manipulation, (B) the same nucleus after manipulation by different pulse energies: 0.54 nJ, 0.73 nJ and 0.91 nJ. In the case of 0.91 nJ pulse energy, the tissue was ablated; but with less energy there was only photobleaching.*

3 Discussion

Fs-nanosurgery can be used to target in the submicron region inside a single cell. Imaging the cell's reaction to the disruption imparts information about the sequences of intracellular processes. In order to observe the viability of the cell in dependence to mitochondria disruption, the cell has to be observed over a longer time period up to one hour with both fluorescence imaging and bright field microscopy imaging. The mitochondria disruption was realized at pulse energies between 0.7 nJ and 1.0 nJ and with a speed of approximately 14 µm/s. The reaction of the cell was observed by comparing the images before and after manipulation.

Typically, a cell changes its volume when it initiates apoptosis which can easily be seen in the bright field microscopy image and in the fluorescence image by an important change of the mitochondria arrangement. Figure 3 shows living endothelia cells whose mitochondria are MitoTracker Orange labeled. The manipulation was realized at pulse energies of 1 nJ. The cuts are between 0.75 µm and 1.26 µm long and 0.7 µm wide.

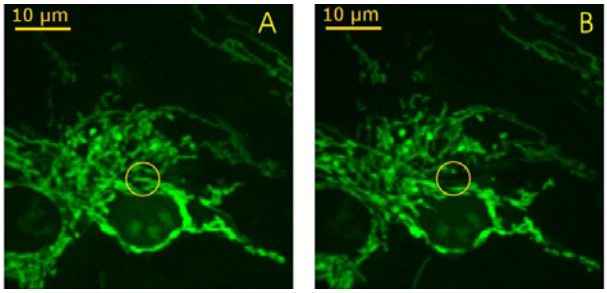

Fig. 3 *MitoTracker Orange labeled endothelia cells before (A) and after (B) manipulation. The mitochondrion highlighted by the circle was disrupted at a pulse energy of 1 nJ.*

4 Conclusion

The presented experiments showed that multiphoton microscopy offers the possibility of imaging single cells and cell organelles. Manipulation of single cell organelles requires the lowest possible cutting energy. This was realized with pulse energies of 0.7 nJ to 1.0 nJ. Equally important is to carefully choose the speed and the irradiation time of the manipulation. Due to the low pulse energy and the low irradiation time the disruption volume is very small. The disruption of a single mitochondrion does not induce cell death.

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