

FEMTOSECOND LASER DISRUPTION OF MITOCHONDRIA IN HELA CELLS

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Studying intact organelles and their function in a living cell is essential to understand cell dynamics. To observe sub-cellular organelles, femtosecond lasers operating in the near infrared region has been utilized in two-photon microscopy. One of the salient features of focusing femtosecond laser pulses is the limited interaction region in three-dimensional space. König *et al.* proposed a novel nanosurgery tool with femtosecond lasers to perform dissection of chromosomes [1] and DNA transfection [2]. Femtosecond laser-based nanoprocessing of cellular structures without compromising the viability of cells has potential applications in cell biology because one can control functional interactions between organelles and inter-cell communication only at targeted locations. A single organelle was completely knocked out without disturbing surface layers and affecting the adjacent organelles or the viability of both plant cells and animal cells [3-5]. In this paper, we present the experimental confirmation of photodisruption of subcellular organelles in a living cell by tightly focusing femtosecond laser pulses inside the cell [6]. HeLa cells were transfected with an EYFP fused with a mitochondria-targeted sequence of cytochrome C oxidase. One-photon fluorescence images of cells were observed by excitation with the Ar⁺ laser at a wavelength of 488 nm, as shown in Fig. 1(a). Femtosecond laser pulses (wavelength 800 nm) at an energy of 3 nJ/pulse were focused with the oil-immersion objective (NA, 1.4) Figure 1(b) shows the confocal image obtained after femtosecond laser irradiation. Fluorescence from the surrounding mitochondria in a region a few microns wide from an irradiated mitochondrion was not observed. Photodisruption of organelles in living cells was confirmed by restaining of organelles after irradiation with femtosecond laser pulses. Impermeability of propidium iodide and the presence of cytoplasmic streaming suggested that the cells are alive after laser irradiation.

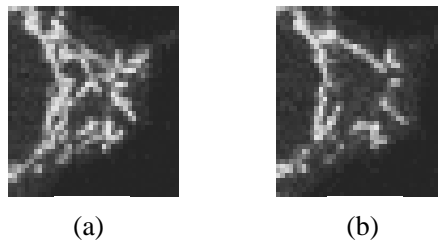


Figure 1: Confocal images of HeLa cell before and after femtosecond laser irradiation. (a) and (b) confocal images of the HeLa cell before and after femtosecond laser irradiation.

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