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ScienceDirect
Applied Surface Science 254 (2008) 3370–3375
applied surface science
www.elsevier.com/locate/apsusc

In situ observation of photo-bleaching in human single living cell excited by a NIR femtosecond laser

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Received 11 May 2007; received in revised form 11 November 2007; accepted 11 November 2007
Available online 23 November 2007

Abstract

The photo-bleaching of single living cells excited by femtosecond laser irradiation was observed *in situ* to study the nonlinear interaction between ultrafast laser pulses and living human breast MDA-MB-231 cells. We conducted a systematic study of the energy dependence of plasma-mediated photo-disruption of fluorescently labeled subcellular structures in the nucleus of living cells using near-infrared (NIR) femtosecond laser pulses through a numerical aperture objective lens (0.75 NA). The behavior of photo-bleached living cells with fluorescently labeled nuclei was observed for 18 h after femtosecond laser irradiation under a fluorescence microscope. The photo-bleaching of single living cells without cell disruption occurred at between 470 and 630 nJ. To study the photo-disruption of subcellular organelles in single living cells using the nonlinear absorption excited by a NIR femtosecond laser pulse, the process of photo-bleaching without photo-disruption provides key information for clarifying the nonlinear interaction between NIR ultrashort, high-intensity laser light and transparent fluorescently labeled living cells.

PACS : 87.17.−d; 52.38.Mf; 42.62.Be

Keywords: Femtosecond laser; Photo-bleaching; Laser internal processing; Living cell; Nucleus; Fluorescence; Photo-disruption

1. Introduction

Due to its wide range of potential applications, the interaction between near-infrared (NIR) ultrashort, high-intensity laser light and transparent material is of great interest in a wide variety of fields [1,2]. In cell biology, microscopy using the nonlinear interaction excited by a NIR femtosecond laser was developed to observe cellular and subcellular structures, and such techniques have been applied to two-photon excitation fluorescence microscopy [3], second-harmonic generation (SHG) microscopy, third-harmonic generation (THG) microscopy, and coherent anti-Stokes Raman scattering (CARS) microscopy [4]. Nonlinear microscopy using NIR femtosecond lasers is appealing because of its high spatial resolution, deep penetration into thick samples, and reduced photon-induced damage [5].

In the meantime, the interaction technique has also been applied to cell manipulation and nanosurgery of living cells and subcellular organelles, as a focused NIR femtosecond laser can selectively modify the targeted cell and subcellular organelle with high spatial resolution on a nanometer to micrometer scale. When intense femtosecond laser pulses are focused on transparent cells, the peak intensity in the focal volume can become sufficiently high to cause nonlinear phenomena, such as multiphoton absorption, tunneling, and avalanche ionization processes [6,7]. Free electrons absorb energy from the electromagnetic field of the laser pulse, leading to laser-induced optical breakdown, and the generation of high-density plasma [8], which ablates the surface or internal structure of cells. The limited heat generation enables precise control of the cellular modification, avoiding peripheral thermal damage [9,10]. Therefore, a femtosecond laser can allow spatially

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0169-4332/$ – see front matter
doi:10.1016/j.apsusc.2007.11.030

selective manipulation of organelles in a targeted cell. Recently, several studies have reported various techniques related to cell processing using NIR femtosecond lasers, including the dissection of chromosomes [11], transfection of DNA [12], disruption of organelles in living cells [13–17], disruption of structures in live embryos [18], dissection of microtubes [19], and disconnection of nematode axons [20].

Although several reports have described the photo-disruption and photo-modification of subcellular organelles in living cells excited with a NIR femtosecond laser, the photo-bleaching of a sub-organelle in a targeted living cell has not been specifically examined. The photo-bleaching phenomenon plays a key role in studies of internal cell processing with fluorescent proteins, regardless of whether the targeted subcellular organelles of a living cell were photo-disrupted. Although Dr. E. Mazur's research group reported the photo-bleaching of the fluorescently labeled nucleus of a fixed cell, a fixed endothelial cell was used to study the pulse energy dependence of cell ablation experimentally [17]. However, a fixed cell is already dead, and does not behave naturally. Thus, the photo-bleaching of a fluorescently labeled sub-organelle has not been demonstrated in a living cell. Therefore, we conducted *in situ* observations of the photo-bleaching of a targeted subcellular organelle in a single living cell to study the nonlinear absorption in cell processing using a NIR femtosecond laser.

In this paper, we report the *in situ* observation of photo-bleaching of fluorescent-labeled proteins in the nucleus of a single living human breast cell excited by a femtosecond laser. The selective photo-disruption of the nucleus of a single living cell without modifying the cell morphology is also demonstrated in living human breast cells using NIR (790 nm) femtosecond (110 fs) Ti:sapphire laser pulses.

2. Experimental

To study the photo-bleaching of a single living cell, we developed a new tool to make *in situ* observations of fluorescently labeled subcellular organelles. It consisted of a fluorescence microscope with a femtosecond laser cell manipulation system, which allowed us to observe the cell morphology and fluorescent images of subcellular organelles in real time. A schematic diagram of the experimental setup for observing the photo-bleaching of sub-organelles in living cells is shown in Fig. 1. A fluorescence microscope system with femtosecond laser cell processing was developed to observe the behavior of targeted sub-organelles with fluorescent proteins in a single living cell using an ultraviolet (UV) mercury lamp.

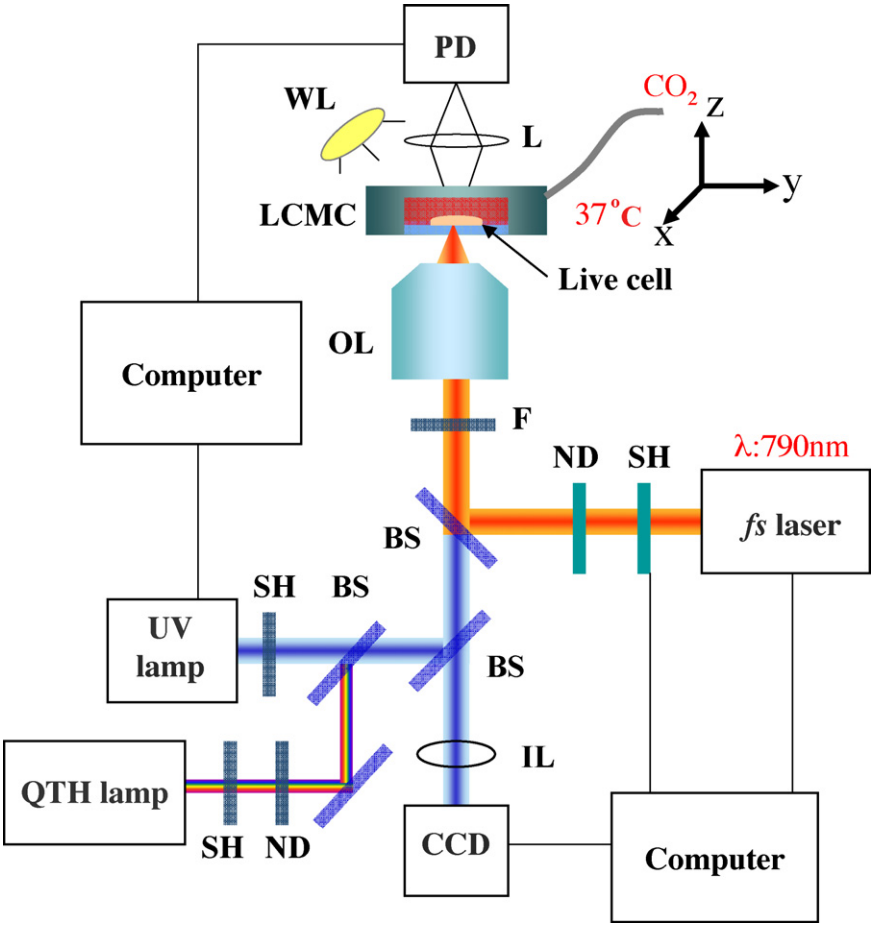

Fig. 1. Experimental setup used to study the photo-bleaching of single living cells. UV lamp: mercury ultraviolet lamp; SH: shutter; LCMC: live cell microchamber; ND: neutral density filter; WL: white light; IL: imaging lens; OL: objective lens; L: lens; BS: beam splitter; QTH Lamp: quartz tungsten halogen lamp; PD: high-sensitivity photodiode; F: fluorescent filter.