Title
Time Resolved Fluorescence Microscopy to Visualize the Dynamics of Optoinjection

ISBN
9781557528070

Authors
Quinto-Su, PA
Heilman, AN
Venugopalan, V

Publication Date
2006

DOI
10.1364/bio.2006.tui51

Time Resolved Fluorescence Microscopy to Visualize the Dynamics of Optoinjection

P. A. Quinto-Su [†‡], A. N. Hellman[*], and V. Venugopalan[†‡]

[†] *Department of Chemical Engineering and Materials Science, University of California, Irvine, Irvine, CA 92697-2575*

[*] *Department of Bioengineering, University of California, San Diego, La Jolla, CA 92093*

[‡] *Laser Microbeam and Medical Program, Beckman Laser Institute, University of California, Irvine, Irvine, CA 92697-3010*

vvenugop@uci.edu

Abstract: We develop a time resolved fluorescence microscopy system to image the dynamics of optoinjection produced by the delivery of a highly focused pulsed Nd:YAG (λ=532nm, 6ns) laser microbeam to the cell membrane of PtK2 cells.

OCIS codes: (140.3440) Laser-induced breakdown; (180.2520) Fluorescence Microscopy

1. Introduction

Pulsed laser microbeams have been used for various applications in cell biology ranging from cellular microsurgery to cell lysis. The application of laser microbeams to achieve molecular delivery has taken two forms: optoporation and optoinjection. Optoporation refers to molecular delivery to a field of cells due to transient membrane damage produced by a laser induced shock wave [1, 2]. Optoinjection refers to targetted molecular delivery to a single cell within a field of cells. This is typically achieved by focusing a low energy pulsed laser microbeam onto the cell surface, thereby transiently permeabilizing the cell membrane [3]. While the capability for optoinjection has been firmly established for several years, the mechanisms and dynamics of the process have not been examined.

2. Method

Figure 1 is a schematic of our experimental setup designed to acquire fluorescent images at a certain delay time following cellular irradiation by a pulsed laser microbeam. The energy of the laser pulse is controlled by rotating a linear polarizer. The pulse is directed through the objective (100x, NA=1.3) of an inverted microscope (Zeiss Axiovert S100) and is focused on the surface of a single cell. We use PtK2 cells grown on a petri dish until they are confluent. The cells are stained with a fluorescent dye (CellTrace, $\lambda_{ex}/\lambda_{em}$ = 577/590 nm, MW=789.55 Da) that is excited by a flashlamp. While CellTrace is membrane permeable, upon entry into the cell it is hydrolysed and retained if the cell possesses an intact membrane. The flashlamp light is combined with the laser pulse on a dichroic so that both are focused on the cell by the microscope objective. Flashlamp illumination is delivered to the sample at a 1-30 μs time delay after the delivery of the Nd:YAG laser pulse. This excites fluorescence to provide images that show the presence of dye within the cell.

3. Results

Figure 2 shows images taken before laser irradiation (-2s), 2, 6, and 14s after the delivery of a single 3.2 μJ laser pulse at time 0s. The plot shows a decrease of the average intensity of the irradiated cell after the pulse that suggests the transport of dye out of the cell. Figure 3 has images of a cell before and after irradiation by a 2.6 μJ laser pulse. The first picture shows the cell before it was irradiated (-2s), the next picture was taken 3.6 μs after the pulse and the rest were taken at 2s intervals. The plot shows the decrease of the average intensity of the dye in the cell as a function of time. The decrease of fluorescent intensity inside

the cell indicates that the cellular membrane has been breached (Figures 2 and 3). However the fact that fluorescence of the cell is retained at long timescales is an indicator that the membrane has been repaired and that the dye is no longer leaking out. This suggests that the cell remains viable. The time scale for membrane repair (on the order of seconds) is consistent with the measurements from other studies [4].

4. Conclusion

We have demonstrated the use of time-resolved fluorescence microscopy to image the dynamics of laser microbeam optoinjection. Experiments are currenty underway to confirm the viability of the cells after irradiation and to analyse the transport dynamics on ns to ms timescales.

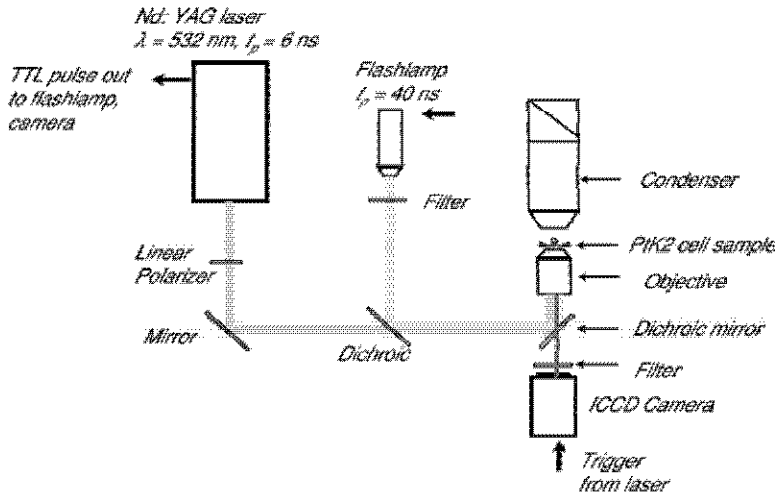

Figure 1: Experimental setup for time resolved fluorescence microscopy. The dicroic in the microscope reflects the 532 nm laser light, the excitation light from the flashlamp (577 nm) and it transmits the fluorescent light from the dye in the cells (590 nm).

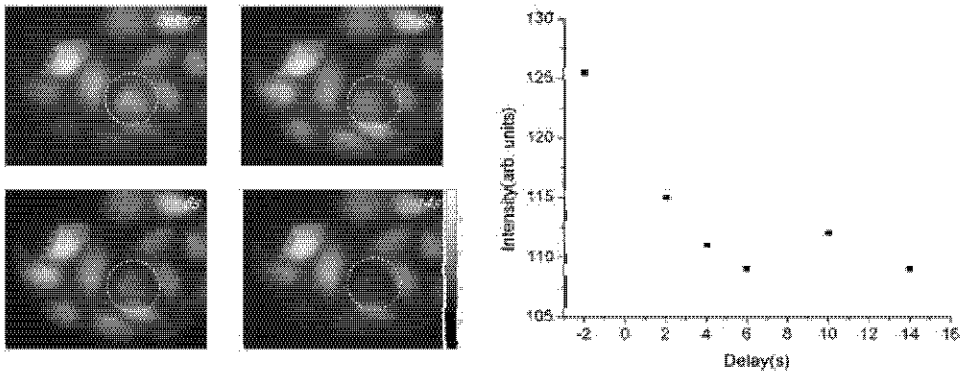

Figure 2: Fluorescent images of a cell before (-2s) and after irradiation (2, 6 and 14s) by a 3.2 μJ laser pulse. The plot shows the average intensity of the irradiated cell as a function of time. The images corresponding to 4, and 10s are not shown.

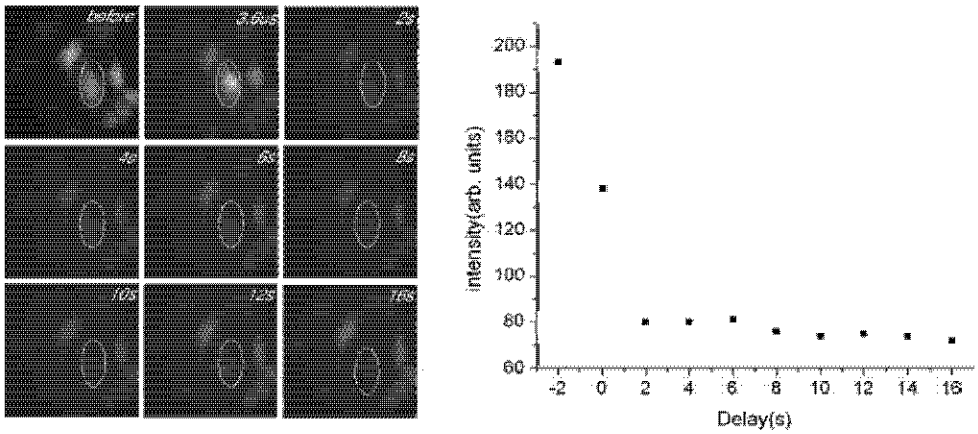

Figure 3: Cell before (-2s), and after irradiation (3.6μs to 16s) by a 2.7μJ laser pulse. The plot shows the average intensity of the irradiated cell as a function of time.

References

[1] I. Turovets, A. Lewis, D. Palanker, H. Gilo, A. Vilenz, J. C. Broder and S. Lewis, "Permeabilizing millions of cells with single pulses of an excimer laser" BioTechniques **15**, 1022-1027 (1993).

[2] J. S. Soughayer, T. Krasieva, S. C. Jacobson, J. M. Ramsey, B. J. Tromberg, and N. Allbritton, "Characterization of cellular optoporation with distance" Anal. Chem. **72** 1342-1347 (2000).

[3] W. Tao, J. Wilkinson, E. J. Stanbridge, M. W. Berns, "Direct gene transfer into human cultured cells facilitated by laser micropuncture of the cell membrane" Proc. Natl. Acad. Sci. **84**, 4180-4184 (1987).

[4] T. Togo, T. B. Krasieva, and R. Steinhardt, "A decrease in membrane tension precedes succesful cell-membrane repair" Mol. Bio. Cell, **11** 4339-4346 (2000).