

Femtosecond near-infrared pulses used for microinjection

Microinjection, the delivery of membrane-impermeable materials into living cells, is an important tool for a variety of applications, including genomics, proteomics, and drug screening and testing. However, many microinjection methods, such as micropipettes, electroporation and biolistics, offer low transfection efficiencies and cell survival rates, cannot be used on certain cell types and are not applicable to single cells. Using UV or 488-nm argon lasers to create an opening can cause cytogenetic and cell membrane damage.

Researchers at Rensselaer Polytechnic Institute in Troy, N.Y., recently used near-infrared femtosecond pulses for microinjection of single cells. Ingrid Wilke, assistant professor in the department of physics, and Robert E. Palazzo, director of the Center for Biotechnology and Interdisciplinary Studies, cultured endothelial cells and *Spisula solidissima* oocytes. These oocytes, which are resistant to standard physical microinjection methods because of a strong vitelline shell outside the plasma membrane, are a model organism used in cell development research.

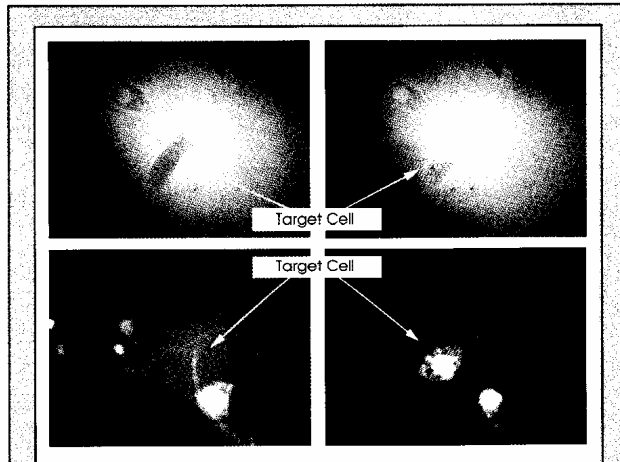
The scientists used 80-fs pulses from a Spectra-Physics Ti:sapphire laser at 800 nm to create a transient artificial pore in a cell's membrane. The laser beam was expanded to the size of the objective aperture by a beam expander and focused on the edge of a single cell with a 40× objective. A shutter with a temporal resolution as short as 1 ms controlled the laser exposure time.

To determine whether a pore was created, they observed the cell's uptake of

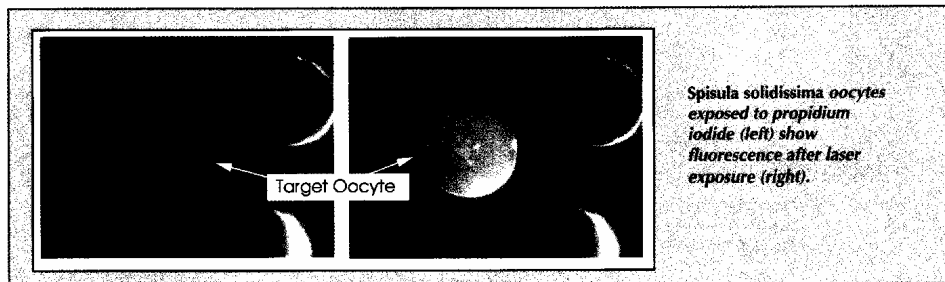
the membrane-impermeable dyes lucifer yellow or propidium iodide with a Nikon inverted microscope and recorded the average fluorescence intensity through time-lapse photography using a Roper Scientific CCD camera. Using the microscope to simultaneously visualize the cell and the approximately 500- to 600-nm laser focal spot — small in relation to the cell, which

measures about 50 to 70 μm — enabled precise control of the pore location.

Graduate student Cheng Peng presented the group's findings at CLEO in May in Baltimore. The researchers found that the laser power required for pore creation was 50 mW. The pore remained open when the laser was on and closed when the laser was turned off. Exactly how long the pore



Researchers recently used femtosecond near-infrared pulses for microinjection of single endothelial cells. Cells exposed to membrane-impermeable dyes lucifer yellow (top) and propidium iodide (bottom) are shown before (left) and after (right) laser exposure. Fluorescence around and at the nucleus of the target cell was observed, indicating that artificial pores were created in the cell membrane.



Spisula solidissima oocytes exposed to propidium iodide (left) show fluorescence after laser exposure (right).

remains open after the laser is turned off is under investigation.

After exposure to 50- to 150-mW pulses, fluorescence was observed inside the cell around and at the nucleus. The average fluorescence intensity increased almost linearly with time, and the scientists observed 100 percent cell survival as judged by the cell's unaltered morphology. Exposure to 150- to 400-mW pulses resulted

in unstable fluorescence, and powers greater than 400 mW destroyed the cells. The technique also successfully created a pore in *Spisula solidissima* oocytes.

Femtosecond near-infrared laser-assisted microinjection will be a useful technique for all types of mammalian cells and especially for microinjection-resistant cells, such as *Spisula solidissima*, Wilke said. The researchers believe that it has

the potential to overcome many of the limitations associated with other microinjection methods because it is compatible with optical methods for cellular analysis, such as two-photon fluorescence and confocal microscopy. It also is applicable to cells in enclosed volumes, such as in tissues or in microfluidic chip analyzers.

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