BIOPHOTONICS

Rotating Line Optical Tweezers Enables Controlled Intracellular Rotation of Microscopic Objects

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There is considerable interest in the use of optical-tweezer-based methods to rotate microscopic biological objects because the ability to orient such objects can aid in the study of the interaction between specific regions of the objects. Most of the techniques used for rotation of objects are limited to absorbing, birefringent or specially fabricated structures and hence are not suitable for rotation of biological objects.[1-3] Recently an interferometric approach suitable for rotation of a trapped biological object has been reported.[4] However, this method has two important drawbacks: poor utilization of the trap laser power due to the loss in the generation of the required interference pattern and high susceptibility of the interference pattern to vibrations. It may also be difficult to use this approach to rotate objects inside biological cells because scattering will degrade the contrast of the interference pattern.

We have demonstrated controlled, continuous rotation of intracellular objects by using rotating line optical tweezers (RLOT); the rotation of the objects was generated by placing a rotating cylindrical lens in the path of the trapping beam.[5] In line optical tweezers, an object lacking spherical symmetry orients itself along the length of the trap. Therefore, when the elliptic trapping beam is rotated, the trapped particle can be rotated around the axis of the laser beam. It may be pertinent to note here that most naturally occurring biological objects do not have spherical symmetry and therefore can be rotated with RLOT. At 25-mW trapping beam power at the object plane, an intracellular object (presumably a calcium oxalate crystal) trapped inside an *Elodea densa* plant cell could be rotated with speeds of up to 4 Hz (Fig.1). Because the limiting speed of rotation is determined by the drag force of the medium upon the rotating objects, the speed of rotation can be increased by increasing the power of the trapping beam. To the best of our knowledge, this is the first demonstration of rotation of intracellular objects in living cells of intact tissue. Rotation of intracellular objects might prove useful in exploring several aspects of developmental biology. For example, a spindle could be reoriented inside a cell during cell division and the delay, if any, in the transition from metaphase to anaphase could be monitored.

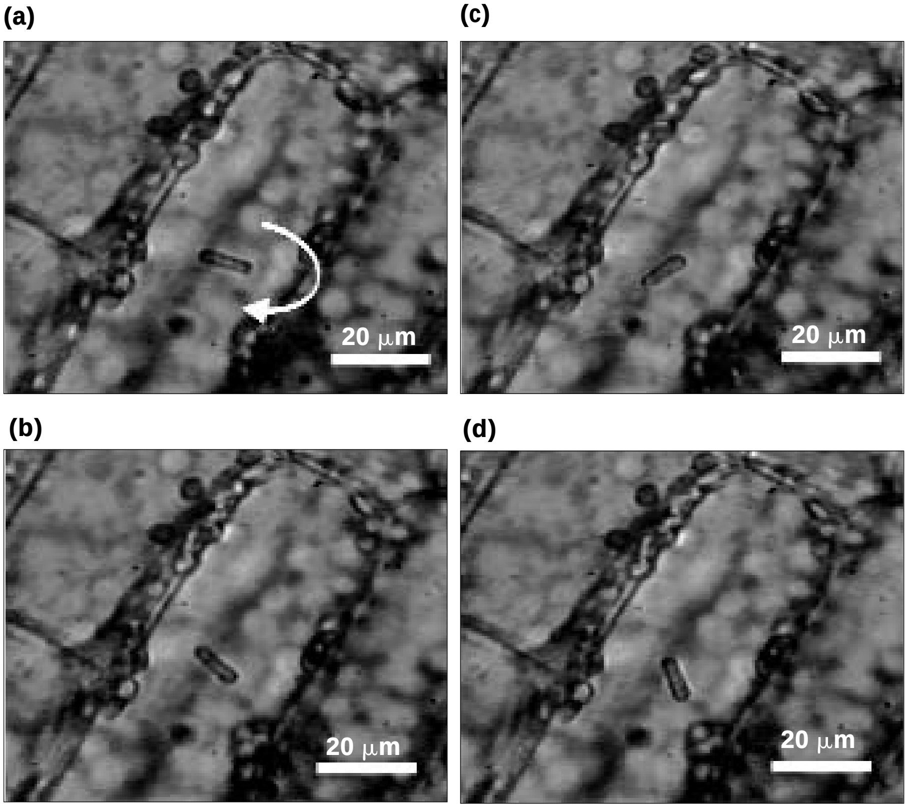

Figure 1. Rotation of an intracellular object inside *Elodea densa* plant cell using the rotating line tweezers. The rod shaped structure was trapped using 25 mW power and rotated at a speed of 4 Hz. The direction of rotation is shown by arrow (a). Clockwise rotation by angles of 45° (b); 145° (c); and 235° (d). All the images were recorded with the same magnification.

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Nanosecond Near-Infrared Laser-Assisted Microinjection Into Targeted Cells

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Laser-assisted microinjection (optoporation) of exogenous material (genes, fluorochromes or photoactivable compounds) into cells is receiving considerable attention in the context of a variety of applications in genetics, cell biology and biotechnology. Compared with conventional techniques, optoporation is more efficient, less tedious and offers the advantage of being usable on cells in suspension as well as on attached cells. Because the constituents of the cell membrane have strong absorption in the ultraviolet (UV) spectral range, lasers in this spectral range were the first to be investigated for optoporation. But the use of UV light raises concerns about damage to cells or even about exogenous biological material being transferred into the cell. Use of lasers of wavelength in the near-infrared region would be more desirable for optoporation because of significantly lower absorption by cellular components in this wavelength range. We have demonstrated the use of a nano-second 1064 nm Nd:YAG laser for microinjection of impermeable fluorochromes as well as transfection of GFP-encoding plasmid into human breast adenocarcinoma (MCF-7) cells.[1]

The Nd:YAG laser beam (17 ns, 10 Hz) was focused by use of a 100 X Plan Neofluor oil immersion objective at the edge of the membrane of the targeted cell, which was suspended in a medium containing impermeable dye merocyanine 540. The energy of the laser pulse at the sample plane was 150 μJ/pulse, resulting in an energy density of ~2.4×10^4 J/cm^2. We observed the dye being injected into the cell through the point of irradiation; its uptake increased with increasing laser exposure. At longer exposure times, we observed the dye fluoresce from the entire cell volume. Optoporation of propidium iodide (PI), another impermeable dye, into MCF-7 cells could also be carried out. However, this required higher energy density (~4.0×10^4 J/cm^2). Because merocyanine 540 has strong two-photon absorption at 1064 nm, we also investigated 1064 nm induced optoporation of propidium iodide in cells whose membranes were stained by merocyanine 540. In this case, optoporation of propidium iodide could be achieved at a lower energy density of ~2.4×10^4 J/cm^2. Further, the fact that even 10 minutes after termination of the irradiation no significant increase in PI fluorescence from the nucleus was observed indicates that membrane integrity of the microinjected cell remained intact. Transfection of GFP plasmid into the cell with the nano-second pulsed laser was also confirmed by monitoring the fluorescence of GFP expressed in the transfected cells (Fig. 1). It may be noted that in our experiments, transfection of GFP plasmid could be achieved by using a single pulse (17 ns), whereas transfection with the near-infrared femtosecond laser required 16 ms.[2]

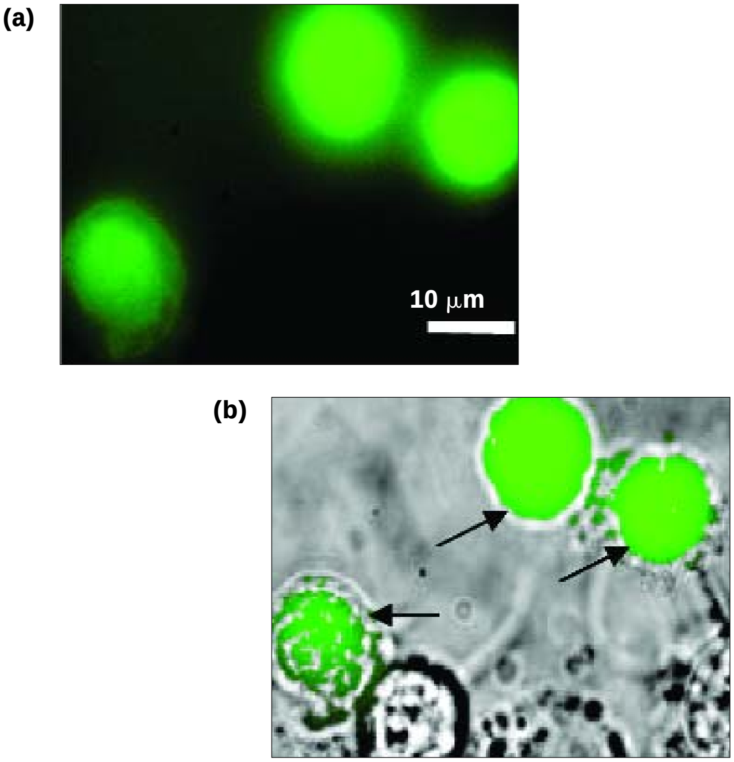

Figure 1. (a) Fluorescence image of MCF-7 cells 24 hours after transfection with green fluorescent protein (GFP) coding plasmid using optoporation with nanosecond 1064 nm Nd:YAG laser beam. (b) Bright field and fluorescence images of cells are overlaid to show targeted cells (shown by arrows). The GFP in transfected cells was excited using 395-440 nm band pass filter and emission was monitored using 470 nm long pass filter. All the images were recorded with the same magnification.

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