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Journal
Experimental Cell Research, 198(2)

ISSN
0014-4827

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Publication Date
1992-02-01

DOI
10.1016/0014-4827(92)90395-o

EXPERIMENTAL CELL RESEARCH 198, 375–378 (1992)

SHORT NOTE

Optical Trapping in Animal and Fungal Cells Using a Tunable, Near-Infrared Titanium–Sapphire Laser

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We have compared two different laser-induced optical light traps for their utility in moving organelles within living animal cells and walled fungal cells. The first trap employed a continuous wave neodymium–yttrium aluminum garnet (Nd-YAG) laser at a wavelength of 1.06 μm. A second trap was constructed using a titanium–sapphire laser tunable from 700 to 1000 nm. With the latter trap we were able to achieve much stronger traps with less laser power and without damage to either mitochondria or spindles. Chromosomes and nuclei were easily displaced, nucleoli were separated and moved far away from interphase nuclei, and Woronin bodies were removed from septa. In comparison, these manipulations were not possible with the Nd-YAG laser-induced trap. The optical force trap induced by the tunable titanium–sapphire laser should find wide application in experimental cell biology because the wavelength can be selected for maximization of force production and minimization of energy absorption which leads to unwanted cell damage.

INTRODUCTION

Laser microbeams have been used for *in vitro* subcellular microsurgery for over 20 years [1, 2]. Recently, this application has been expanded to the manipulation of cells and organelles by light-induced optical forces [3–9]. Although generally promising, the utility of this approach may be limited because of light-induced thermal or photochemical damage to the biologic systems. To determine if this exciting new technique will have major application in biology, we have compared a new titanium–sapphire laser-induced optical light trap with the commonly used neodymium–yttrium aluminum garnet (Nd-YAG) trap for their utility in moving organelles within living animal cells and walled fungal cells. In previous work, we have shown that optical trapping can be used to micromanipulate chromosomes and to study noninvasively the mitotic spindle of living cells [6]. The optical force was generated by a continuous wave, Nd-YAG laser at a wavelength of 1.06 μm. Trapping had to be performed using a low laser power to prevent cell damage most likely caused by the local generation of heat. This problem severely limited the amount of force that could be generated within the cell and, therefore, limited the ability to study the mitotic spindle and manipulate chromosomes.

The titanium–sapphire trap can be readily tuned to wavelengths between 700 and 1000 nm, where the absorption of light energy by water should be reduced 10-fold relative to that of the Nd-YAG laser operating at 1.06 μm [10]. We assumed that this lower absorption by water would result in less heat production and damage to the cell. Thus, a larger force could be generated without damage to the cell. In addition, the trap should be stronger compared to the Nd-YAG laser for a given laser power since more of the energy would be available for force production, and the shorter wavelength can be focused to a smaller spot, producing a larger intensity gradient corresponding to a greater force.

In addition to manipulation of organelles within cultured animal cells, this approach is especially promising for use with walled cells that are under turgor pressure because it does not puncture the wall and disturb the turgor. In this paper, we report on the use of laser-induced optical light traps to manipulate organelles within living, hyphal cells of the fungus *Nectria haematococca* [11].

MATERIALS AND METHODS

The titanium–sapphire laser (Coherent Model 899) was substituted for the Nd-YAG laser in a previously described system (Fig. 1) [12]. For the work described herein, the focusing lens between the dichroic reflector and the microscope was removed. A 6.3× projection lens was

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0014-4827/92 $3.00

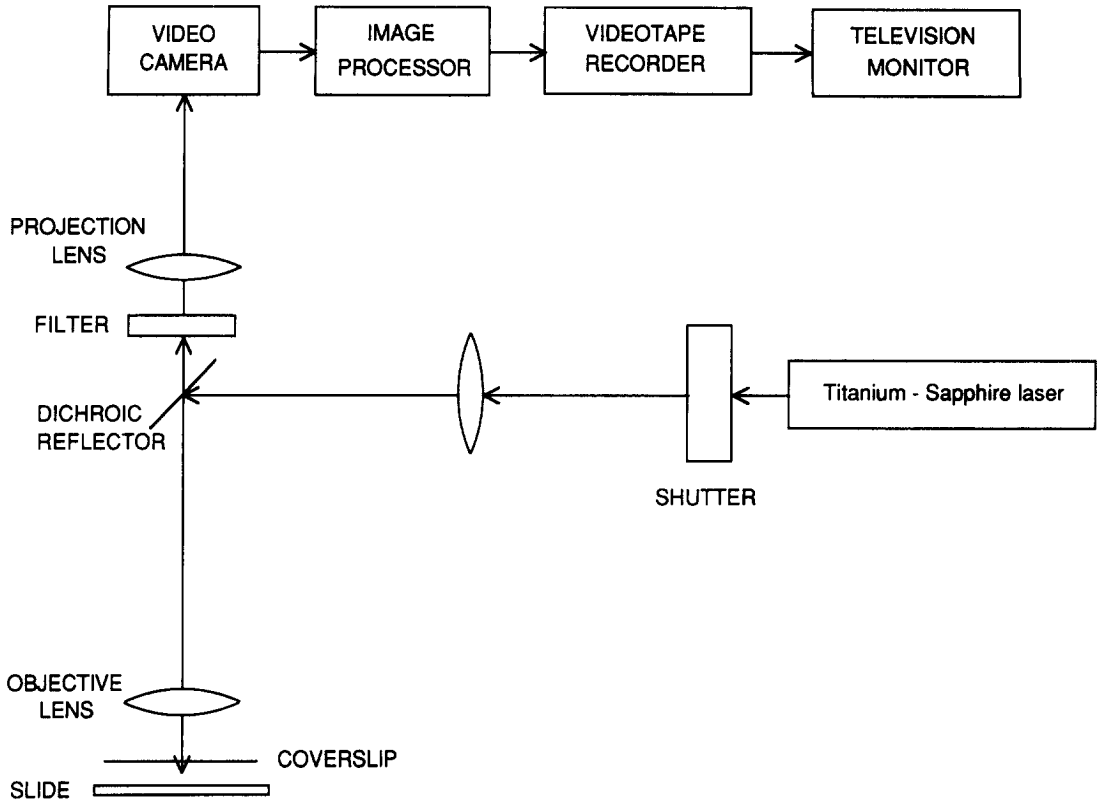

FIG. 1. Schematic diagram of the trapping system.

placed between the dichroic reflector and the video camera. Also, a 15-cm converging lens was placed between the laser and the dichroic reflector; it was translated along the axial direction so that the trapped particle was in focus in the video image. The objective was a Zeiss 100× neofluar, having a numerical aperture of 1.3. The digitized video images were produced by real-time background subtraction [13] and frame averaging [11].

Potorous tridactylis (PTK_2) cells were grown in Rose tissue culture chambers as described previously [6]. They were kept at 37°C during the experiments. The laser was tuned to a wavelength of 700 nm. For these cells, the laser power was measured to be 460 mW after the microscope objective using a Coherent Model 210 power meter. Hyphae of the fungus *N. haematococca* were grown on medium-coated slides as described previously [11]. The medium consisted of 2% yeast extract, 0.2% glucose, 1% Gelrite, and 12% gelatin. The laser was tuned to 760 nm and operated at 90–150 mW of power for the fungal cells.

RESULTS AND DISCUSSION

In this study, we used a titanium–sapphire laser at much higher power levels (producing greater forces) than the Nd-YAG laser without causing apparent cell damage. When light at a wavelength of 700 nm and at power levels of 200–500 mW transmitted through a 100× objective were used, the chromosomes, regardless of their location on the mitotic spindle, could be pulled or rotated with ease, as shown in Fig. 2. When the optical trap was repositioned on the other side of the chromosome, the trapped chromosome could be moved back to its original position, and mitosis continued normally. The ability to manipulate chromosomes with this degree of facility was not possible using the Nd-YAG laser at 1.06 μm. This indicates that the shorter wavelength, titanium–sapphire laser may be a more powerful optical trapping tool because it can be used to produce more force with less secondary effects due to energy absorption by water and other natural chromophores.

In initial experiments with the fungal cells, we used a trap induced by the 1.06-μm Nd-YAG laser [6]. This trap, when operated at relatively low laser power levels (<340 mW after the objective), moved nucleoli around within interphase nuclei and pulled lipid bodies around in the cells with ease and without causing visible damage to the cells or organelles. It also pushed aside the bright vacuoles that regularly occur in these cells. Increasing the laser power to >370 mW after the objective increased the force of the trap, but it also caused several kinds of visible damage, presumably due to the local generation of heat: mitochondria became swollen and rounded up, spindles were broken or collapsed when targeted, and organelles exhibited increased Brownian motion after several minutes of exposure of a cell to the trap. These results, although encouraging in some respects, pointed out the need for a trap that would produce considerably less heat in the fungal cells.

Our assumptions regarding the titanium–sapphire

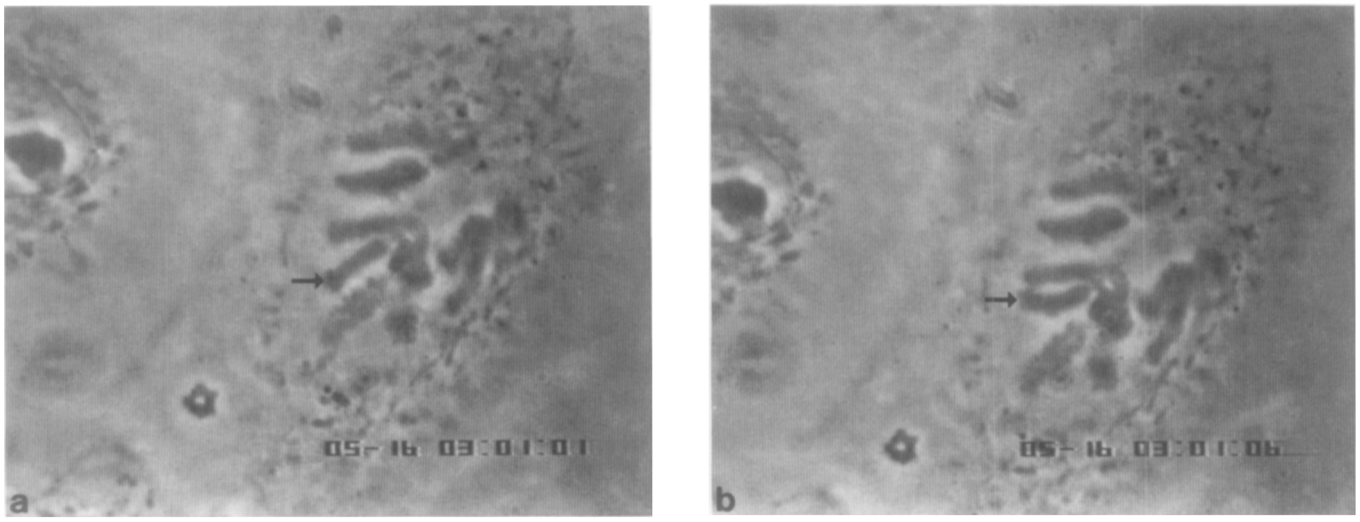

FIG. 2. (a) A chromosome arm (arrow) prior to being subjected to the titanium–sapphire optical trap. The laser was tuned to 700 nm and the power after the objective was 460 mW. (b) Immediately after application of the optical trap, the chromosome is pulled 2.5 μm.

laser apparently were correct as this new trap was much stronger than the previous one at laser power levels that caused no apparent damage to the cells or organelles. We were able not only to move nucleoli around within interphase nuclei but also to separate and pull them far away from the nucleus and even displace the nucleus (Fig. 3). Moreover, Woronin bodies [14] could be removed from the septum, either permanently or temporarily (Fig. 4), a maneuver that was impossible with the Nd-YAG laser trap. These micromanipulations for the first time demonstrated the role of the spindle pole body in maintaining nuclear position at interphase (Fig. 3) and the tethering of Woronin bodies to specific sites on the septum (Fig. 4). With the titanium–sapphire laser trap, we also trapped mitochondria with ease and dragged them long distances (>10 μm) through the cell and translocated spindle pole bodies across interphase nuclei (both maneuvers could not be done with the Nd-YAG laser trap). At the highest laser power (90 mW at the objective focal point) that would not cause visible organelle damage within several minutes, mitochondria and lipid bodies within 4 μm of the trap center were

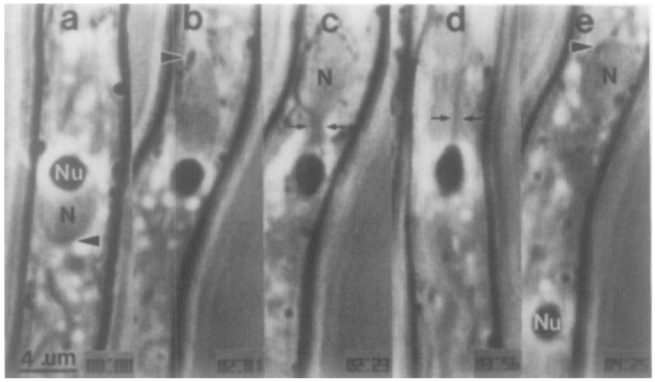

FIG. 3. The titanium–sappire laser can create a strong, near-visible force trap without causing obvious damage to a living, hyphal cell of the fungus *Nectria haematococca*. A nucleolus (Nu) in an interphase nucleus (N) is caught in the trap (a) and pulled to the lower end of the nucleus where continued pulling elongates the nucleus and displaces it about 10 μm down the cell (b). Further pulling on the nucleolus (c) causes the nuclear envelope to collapse (arrows) behind it. It is then possible to pull the nucleolus more than 70 μm down the cell from the nucleus (d) while stretching the intact nuclear envelope to a fine thread (arrows). Finally, the nucleolus escapes the trap and springs back rapidly to within about 20 μm of the nucleus (e). Meanwhile, the spindle pole body (arrowheads) has apparently resisted the pulling force, becoming and remaining situated at the opposite end of the nucleus (a, b, and e). Elapsed time (in min:sec) is shown at the bottom of each frame. The laser was tuned to 760 nm at a power level of 150 mW.

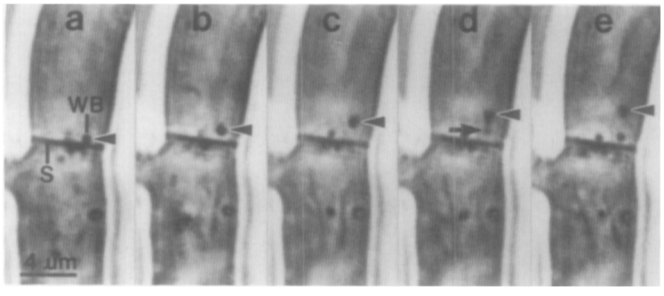

FIG. 4. The titanium–sapphire laser trap can be used to pull Woronin bodies (WB) from the septum (S). A Woronin body is caught in the trap (arrowheads, a) and pulled away from the septum (b and c). The Woronin body then escapes from the trap (arrow, d) and snaps back to its previous site on the septum (e). Other trapped organelles remain in the trap (d and e). Because the Woronin body escaped the trap at a distance of 2 μm from the septum and sprang back to its original position, we infer that it was tethered to a specific site on the septum by an invisible, elastic filament. The laser was tuned to 760 nm at a power level of 90 mW.

suddenly drawn into the trap when it was switched on. The trap was so strong that a group of 5–10 mitochondria in an area would be drawn into it and condensed to a relatively small, dark mass only to spring back to their original size and distribution when the trap was switched off. Not only was there no swelling of mitochondria, but mitotic spindles were also unaffected, in contrast to the damaging side effects of the Nd-YAG laser trap.

Despite the success and obvious advantages of the new titanium–sapphire laser trap, there are several factors that will limit its usefulness. These factors include damage to organelles (e.g., mitochondrial swelling) at high power levels, inadvertent trapping of several-to-many organelles at the same time (which can reduce the force applied to any single organelle in the trap or even cause displacement of the desired organelle from the trap), and variation among different organelle types in their response to the trap (in the fungal study: lipid bodies > nucleoli > mitochondria > chromosomes). Thus, the most suitable specimens will be well-separated, phase-dark organelles having discrete borders and lying in a phase-light homogeneous background milieu, e.g., chromosomes on the mammalian spindle [6] and interphase nucleoli. Despite the limitations mentioned, the advantages of the new laser trap should make it preferable for many applications, and the results clearly demonstrate the utility of optical trapping in fungal and animal cell biology.

Received August 19, 1991

We thank Steve Cheng for technical assistance. This work was supported by funds from the NIH RR01192 (M.W.B.), ONR N00014-90-C-0029 P05 (M.W.B.), the NSF DCB-8916338 (J.R.A.), and the USDA (Hatch Project NYC-153432, J.R.A.).

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