Optical trapping and surgery of living yeast cells using a single laser

Jun Ando,[1,2] Godofredo Bautista,[1] Nicholas Smith,[2] Katsumasa Fujita,[2] and Vincent Ricardo Daria[1,a)]
[1]*National Institute of Physics, University of the Philippines, Quezon City, 1101 Philippines*
[2]*Department of Applied Physics, Osaka University, 2-1 Yamadaoka, Suita, Osaka 565-0871, Japan*

(Received 8 June 2008; accepted 12 September 2008; published online 20 October 2008)

We present optical trapping and surgery of living yeast cells using two operational modes of a single laser. We used a focused laser beam operating in continuous-wave mode for noninvasive optical trapping and manipulation of single yeast cell. We verified that such operational mode of the laser does not cause any destructive effect on yeast cell wall. By changing the operation of the laser to femtosecond-pulsed mode, we show that a tightly focused beam dissects the yeast cell walls via nonlinear absorption. Lastly, using the combined technique of optical microsurgery and trapping, we demonstrate intracellular organelle extraction and manipulation from a yeast cell. The technique established here will be useful as an efficient method for both surgery and manipulation of living cells using a single laser beam. © *2008 American Institute of Physics.* [DOI: 10.1063/1.2999542]

I. INTRODUCTION

Applications of near-infrared (NIR) lasers in biomedical research have grown rapidly since the energy at this region in the electromagnetic spectrum is not enough to initiate light damage on cells and tissues. However, recent developments in ultrafast technology have extended the applications of NIR lasers to allow for nonlinear processes thereby initiating cellular surgery and stimulation, three-dimensional nanoscale processing of intracellular components, cytoskeleton, neuron connections, cellular plasma membrane, and more.[1–8] Biological phenomena such as Ca^{2+} wave generation, gene transfection, and nerve regeneration have also been observed by the careful application of ultrashort light pulses to cause selective destruction of intracellular Ca^{2+} storing organelles,[2,3] localized tiny perforation of cellular plasma membrane,[4] and nanoscale axotomy.[5]

The use of NIR lasers in continuous-wave (cw) mode for optical trapping (or optical tweezers) has also been demonstrated as a contact-free and a noninvasive tool for cell and organelle manipulation.[9,10] Tightly focused NIR laser beams generate a gradient force, which confines biological objects in the vicinity of the laser focus, and by scanning the beam, three-dimensional optical trapping and manipulation can be performed. Currently, optical tweezers are widely used for biomedical research such as the measurement of kinesin molecular movement,[11] drug screening,[12] cell sorting,[13] and investigation of DNA mechanics.[14]

Organelle extraction using polarization-shaped optical vortex traps has recently been demonstrated. Jeffries *et al.*[15] showed optical trapping and extraction of intracellular lysosomes through a B-lymphocyte's cellular membrane, which can be applicable for isolating and manipulating single organelles from a heterogeneous population of healthy and defective organelles in a cell undergoing organelle malfunction. Their succeeding work on the development of a droplet nanolaboratory platform allows selected organelle encapsulation within a femtoliter-volume aqueous droplet.[16] Shelby *et al.*[17] demonstrated optical extraction of intracellular organelles from living cells using two lasers: a nitrogen nanosecond pulsed laser operating in the ultraviolet region for membrane surgery and a neodymium doped yttrium aluminum garnet NIR laser for optical trapping. The same method was used for optical delivery of microscopic objects into plant callus cells[18] and selective fusion of myeloma cells.[19]

In this work, we report optical trapping and surgery of living yeast cells using a single NIR laser operated in both cw and femtosecond-pulsed mode. We show that cells can be noninvasively trapped with a focused NIR laser operating in cw mode. Furthermore, we show that by switching the operation of the laser to femtosecond-pulsed mode, the walls and intracellular organelles of the yeast cells can be disrupted and precise cellular surgery can be performed. Lastly, we report intracellular organelle extraction and manipulation from within a single yeast cell, using the combined technique of laser surgery and trapping.

II. EXPERIMENTAL PROCEDURE

The schematic of the experimental setup for optical trapping and surgery is shown in Fig. 1. We use a titanium:sapphire laser (Tsunami, Spectra Physics) pumped with a $Nd:YVO_4$ (Millennia V4, Spectra Physics). The wavelength of the Ti:sapphire laser is centered at 780 nm and has a repetition rate of 80 MHz when operated in femtosecond-pulse mode. The laser is introduced to a custom-built inverted laser-scanning microscope and brought to focus inside the sample using a water immersion objective lens ($60\times/1.20$, Olympus). The microscope setup includes a dichroic mirror to direct the NIR laser to the objective lens while allowing images of cells at the focus of the same objective lens to be viewed on a charge coupled device camera. The laser focus can be scanned in the *x-y* plane using two

a)Author to whom correspondence should be addressed. Electronic mail: vdaria@nip.upd.edu.ph.

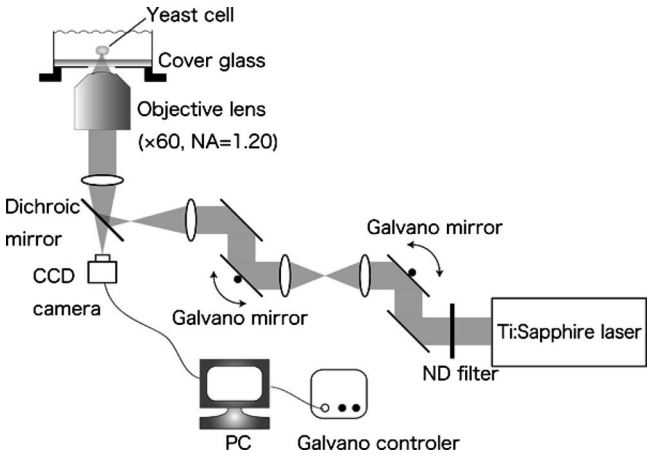

FIG. 1. Experimental setup for laser trapping and surgery of living yeast cells.

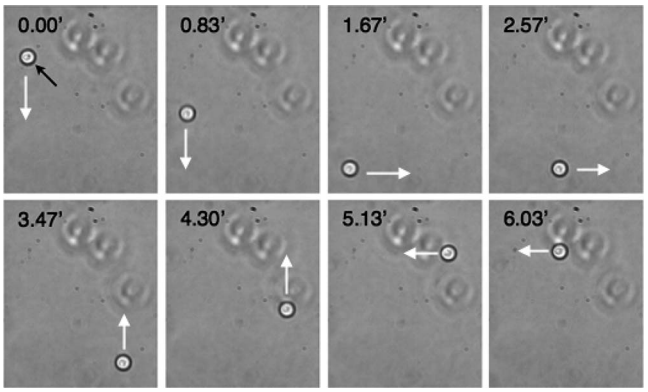

FIG. 2. Sequential bright field images of an optically manipulated single yeast cell using scanning cw Ti:sapphire laser (supplementary movie_01).[20] White arrows indicate the beam path.

galvano mirrors. Time-lapse sequences of bright field images are recorded. Image acquisition and control of the scanning mirrors are achieved using a computer. The software to control the instrumentation is written in LABVIEW (National Instruments). We used yeast *Saccharomyces cerevisiae*, which are grown in glucose yeast peptone medium (20 g/l glucose, 10 g/l peptone, and 5 g/l yeast extract with distilled water) at room temperature (25–27 °C).

III. OPTICAL MANIPULATION OF SINGLE YEAST CELLS

Using the experimental setup shown in Fig. 1, we succeeded in trapping and manipulating a single yeast cell with a Ti:sapphire laser operating in cw mode. Figure 2 shows sequential bright field images of an optically manipulated yeast cell. The targeted cell was optically trapped using the tightly focused laser beam with 10 mW average laser power, measured after the objective lens. Using appropriate programming to move the scanning mirrors, the beam was moved along a square consequently transporting the cell with the beam (supplementary movie file).[20] Even after several minutes of manipulation, there was no significant disruption of the cell such as shrinkage or cell wall dissection. This indicates that the energy of the laser in cw mode is not sufficiently absorbed by the cell walls to cause visible disruption or other observable effects on the cell viability.

We verified the cell viability and growth under the laser trap by observing the growth of an optically trapped yeast cell. Saccharomyces cerevisiae is known to grow via the reproductive process known as budding. Figure 3 shows bright field images (top) and a graphical illustration (below) of a budding yeast cell with and without the presence of an optical trap using a focused cw mode Ti:sapphire laser. In this experiment, we used the same laser power, set at 10 mW, as measured after the objective lens. The observation started with no incident laser (laser off) and showed two yeast cells that were horizontally oriented. One of the cells was budding in the upward direction indicated by the black arrow. When the focused laser was incident, the trapping force oriented the yeast cell perpendicular to the glass substrate as graphically illustrated. The laser was incident for 2 h (120 min) and showed no effect on the cell viability as indicated by the increase in the size of the yeast cell bud. A second bud was observed in an out-of-focus plane as manifested by a shadow in the bright field image. When the laser was turned off, the cells were again oriented in the horizontal position, clearly showing the second bud.

IV. OPTICAL SURGERY OF YEAST CELLS

Using the same setup, we set the operation of the Ti:sapphire laser to mode-lock (ML) femtosecond-pulse mode.

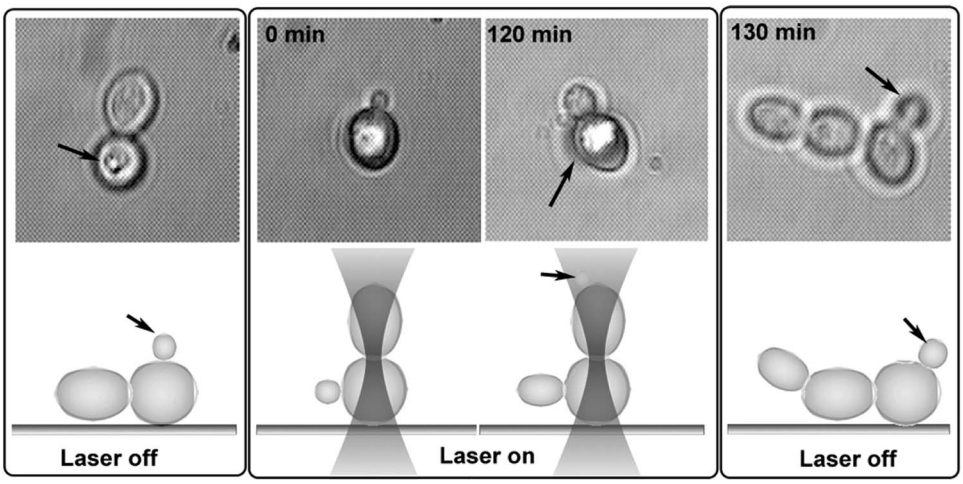

FIG. 3. Bright field time-lapse images of optically trapped yeast cell growth using cw Ti:sapphire laser at 10 mW of laser power.

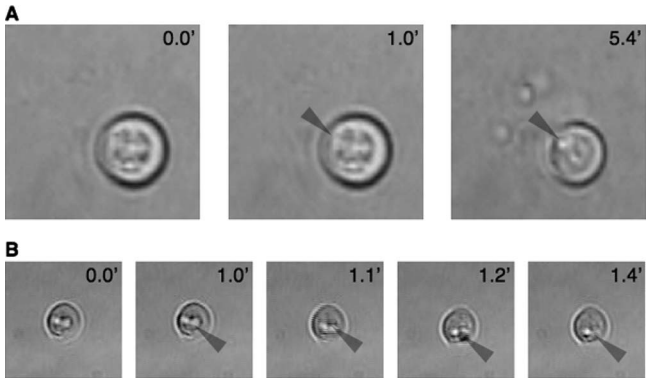

FIG. 4. (a) Sequential bright field images of yeast cell wall dissection using femtosecond-pulsed Ti:sapphire laser irradiation. Gray triangles indicate the position of the laser focus (supplementary movie_02).[20] (b) Sequential bright field images of yeast's intracellular organelle disruption using femtosecond-pulsed Ti:sapphire laser irradiation. Gray triangles indicate the position of the laser focus (supplementary movie_03).[20]

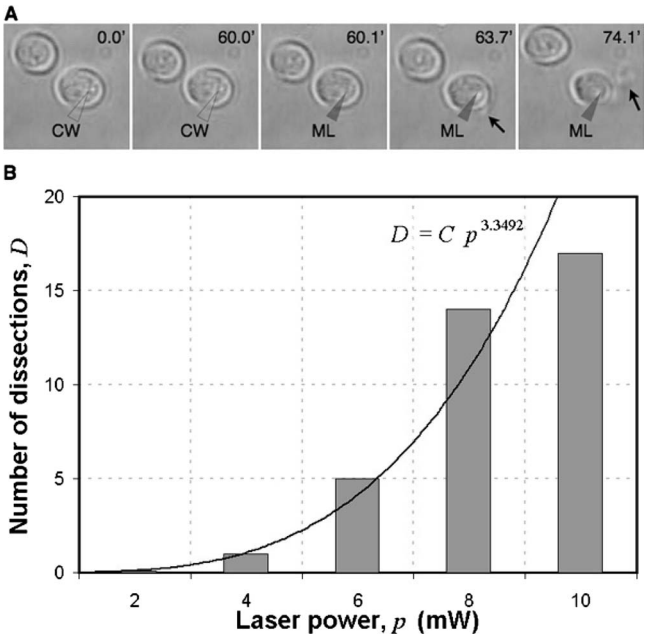

FIG. 5. (a) Sequential bright field images of yeast cells with Ti:sapphire laser irradiation. Unfilled and filled triangles indicate the position of the laser focus in cw and femtosecond-pulse operation mode operation, respectively. (b) Number of dissections of yeast cell wall as a function of laser power. Exposure time was fixed to 5 s and 20 cells were irradiated per data point.

Here, we observe laser induced cell wall dissection resulting in efflux and selective breakup of intracellular organelles. Figure 4(a) shows sequential bright field images of yeast cell wall dissection using a Ti:sapphire laser operating in ML mode. The black triangle indicates the position of the laser focus. In this experiment, the laser power was set to provide 10 mW at the focus. Around 4 s after laser irradiation, the cell wall was dissected resulting in efflux of some intracellular organelles and more efficient visualization of the organelles inside the cell (supplementary movie file).[20] By moving the focus inside the cell, selective breakup of organelles in a yeast cell is shown in Fig. 4(b). The intracellular organelles were initially attracted toward focus (indicated by black triangle), and as the integrated power accumulates after 1.2 s of illumination, the organelle broke apart (supplementary movie file).[20]

We further verify the dependence on the operational mode of the laser on the induced yeast cell wall dissection, when operating the Ti:sapphire laser in cw and femtosecond-pulse mode. Figure 5(a) shows sequential bright field images of yeast cells under cw and femtosecond-pulsed laser irradiation. The laser focus was fixed on the yeast cell wall with 10 mW of average laser power. From 0 to 60 s, the Ti:sapphire laser was operated in cw mode and from 60 s the Ti:sapphire laser was switched to femtosecond-pulse mode. Immediately after switching the laser mode, the cell wall was dissected resulting in efflux of organelles. This shows that laser dissection of the yeast cell wall occurs only when the Ti:sapphire laser is operated in femtosecond-pulse mode and indicates that the yeast cell wall dissection is due to nonlinear (multiphoton) absorption. In 2005, Sacconi *et al.*[21] compared cw and femtosecond-pulsed Ti:sapphire laser for laser surgery of yeast mitotic spindle, and they also concluded that only femtosecond-pulsed Ti:sapphire laser can cause yeast mitotic spindle dissection at average power levels of up to 4 mW. Moreover, Vogel *et al.*[22] investigated the working mechanisms of femtosecond laser nanoprocessing and concluded that ultrashort laser pulses initiate multiphoton ionization. The ionization induces plasma formation and results in a dramatic, transient, and local increase in the absorption coefficient at the focus, to a level significantly higher than the linear absorption coefficient. We can similarly characterize optical dissection of the cell walls and resulting cavitation as nonlinear absorption effects due to their occurrence only in femtosecond-pulsed mode irradiation.

Next, we provide proof of nonlinear absorption by taking the histogram relating the number of induced cell wall dissections as a function of input laser power of the NIR femtosecond-pulsed laser. In the experiment, the average laser power was varied from 2 to 10 mW, measured just after the microscope objective. The focus position was set on the cell wall and the exposure time was set to 5 s. Figure 5(b) shows the number of cell wall dissections as a function of average laser power. We directed the focus on 20 cells for each power setting and cell wall dissections were confirmed by the resulting efflux of intracellular organelles. At power levels equal or less than 2 mW, no cell wall dissection was detected. Increasing the power to 6 mW allowed 5 dissections over 20 trials on different cells. Further increasing the average power to 8 and 10 mW allowed 14 and 17 cell wall dissections, respectively. On the other hand, there were no observed dissections with cw mode of Ti:sapphire laser even with 10 mW of average laser power at the focus. The plot for the number of dissections shows a nonlinear curve indicating that the optical surgery of the cell wall is based on multiphoton absorption. Moreover, the power relationship measured here is an indirect measurement of the various absorption processes, and it is therefore difficult to break down the effects into established principles. The processes involve the combination of multiphoton absorption in the cell wall and multiphoton ionization of water as well as linear absorption. The final power relationship also contains some complex re-

lationships such as the effective size of the focal spot being larger at higher powers creating stronger shearing effects during bubble expansion and collapse, thereby increasing the area of dissection and cavitation.

In 1997, König *et al.*[23] studied the influence of NIR femtosecond Ti:sapphire laser irradiation on cell reproduction. Chinese hamster ovary cells were exposed to focused scanning beam of 800 nm femtosecond Ti:sapphire laser with the focal spot scanned ten times over each cell. In their study, the laser power threshold of cloning inhibition (i.e., a decrease in cloning efficiency from 100% to 0%) was 2 to 6 mW of laser power, and complete cell destruction was observed at mean powers of 10 mW. They concluded that extremely high photon density at the focal spot (10^{32} photons cm^{-2} s^{-1} with 10 mW of mean laser power) might induce destructive intracellular plasma formation. The laser power threshold of cloning inhibition shows good agreement with yeast cell wall dissection, thus we can assume that cloning inhibition may have been caused by the kind of laser induced cell membrane destruction that we have observed here.

While it may be considered that the damage through the cell walls is caused primarily by thermal effects, the major contributing factor to the cell wall dissection is photochemical. The work by Vogel *et al.*[22] also explains the various processes in water and soft water-based biological targets with particular relevance to laser ablation of cells. The photochemical process in the cell wall dissection is driven by the buildup of structural changes caused by free electrons in and around the focal zone during the laser exposure. At high numerical apertures, the gradient between any deposited energy that manifests as heat in the focal zone and the surrounding areas are such that thermal accumulation does not occur significantly. In general, thermal accumulation occurs at energy densities above which photochemical effects can already occur. This means that although it is possible to create thermal effects in the sample, other disruptive forces are acting on the sample at lower energy densities, particularly for relatively long exposure times such as those used in the experiments reported here. When looking at threshold-type events such as the onset of cell wall dissection, low-density plasma and general photochemical effects dominate the interaction.

At high laser repetition rates, dissection on the cell walls can occur by low-density plasmas formed at the laser focus which can then decompose chemical bonds in surrounding molecules. Particularly for dissection that occurs by a train of femtosecond pulses, where individual pulse energies are low, the dissection occurs as a buildup of modifications of the sample, without significant thermal or thermoelastic stress effects. In our experiment, we used pulse energies of approximately 0.04–0.10 nJ and laser exposures of up to 5 s (corresponding to around 400×10^6 pulses). Hence, each pulse energy is well below the threshold to produce significant thermal effects from a single pulse, and the total exposure time is long enough that any thermal rise occurring due to individual pulses can diffuse away without building up at the laser focus.

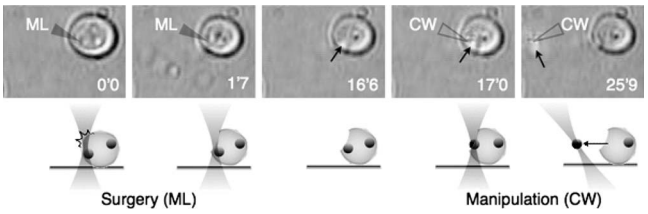

FIG. 6. Intracellular organelle extraction and manipulation using the combined technique of optical surgery and trapping. Unfilled and filled triangles indicate the position of the laser focus of the cw and femtosecond-pulsed Ti:sapphire laser, respectively. Black arrows indicate targeted intracellular organelle (supplementary movie_04).[20]

V. COMBINING OPTICAL TRAPPING AND SURGERY FOR EXTRACTING CELL ORGANELLES

Lastly, we performed experiments demonstrating extraction of cell organelles using the combined technique of laser surgery and trapping. Figure 6 shows sequential bright field images of extraction and manipulation of yeast cell organelles (supplementary movie file[20]). From 0 to 4 s, the Ti:sapphire laser in femtosecond-pulse mode was focused on the cell wall at an average laser power of 10 mW at the laser focus in order to dissect the cell wall. To avoid excessive destruction of the cell wall, the laser was only incident for 4 s. Dissection resulted in efflux of organelles from the cell. From 17 s, the Ti:sapphire laser operation was changed to cw mode at 10 mW of average laser power, and the laser focus was scanned from right to left direction as shown in Fig. 6. A single intracellular organelle was successfully manipulated and extracted through the area of the cell wall opened by laser irradiation.

Organelle extraction with combined technique of optical trapping and surgery, which we presented in our paper, will be useful for the organelle analysis of plant cells and yeast cells because it enables us to extract organelles even if there is rigid cell wall. Especially since yeast cells are treated as model organisms of eukaryotic cells, organelle analysis of yeast cell will be useful for the investigation of organelle malfunction causing human disease. In 2003, Feuermann *et al.*[24] reported that the yeast cell can be used as a model organism for mitochondrial malfunction causing neurodegenerative disease and concluded that mitochondrial elongation factor returns defective phenotypes to normal. Hence, manipulation and single yeast cell analysis allow for rapid assays of potential genetic targets and cure.

VI. CONCLUSION

A single Ti:sapphire laser operated in cw and femtosecond-pulse mode can be used for both optical trapping and surgery of living yeast cells, respectively. The energy from the cw NIR laser is low and not enough to prevent cellular damage, while the high intensity at the focus allows for sufficient trapping force to immobilize the cells. When the laser is switched to femtosecond-pulsed mode, the nonlinear absorption allows dissection of the cell walls. We have also shown nonlinear dependence of the probability of cell wall dissection as a function of input average power. Lastly, we demonstrated that the combined technique of laser surgery and trapping can be used to realize a simple method of

selective intracellular organelle extraction and manipulation. To the best of our knowledge, this is the first report that demonstrates the practical application of a single laser for organelle extraction using optical surgery and optical manipulation.

ACKNOWLEDGMENTS

The project has been funded by the Philippine Council for Advanced Science and Technology Research and Development (PCASTRD). J. Ando acknowledges the support of the Japan Student Services Organization (JASSO) for the short-term student exchange promotion program. The authors also thank APL Hilario for the technical advice and support on the image acquisition and scanning control software.

[1] K. König, I. Riemann, P. Fischer, and K.-J. Halbhuber, Cell. Mol. Biol. (Paris) **45**, 195 (1999).
[2] N. I. Smith, K. Fujita, T. Kaneko, K. Katoh, O. Nakamura, S. Kawata, and T. Takamatsu, Appl. Phys. Lett. **79**, 1208 (2001).
[3] S. Iwanaga, T. Kaneko, K. Fujita, N. Smith, O. Nakamura, T. Takamatsu, and S. Kawata, Cell Biochem. Biophys. **45**, 167 (2006).
[4] U. K. Tirlapur and K. König, Nature (London) **418**, 290 (2002).
[5] M. F. Yanik, H. Cinar, H. N. Cinar, A. D. Chisholm, Y. Jin, and A. Ben-Yakar, Nature (London) **432**, 822 (2004).
[6] H. Hirase, V. Nikolenko, J. H. Goldberg, and R. Yuste, J. Neurobiol. **51**, 237 (2002).
[7] N. Shen, D. Datta, C. B. Schaffer, P. LeDuc, D. E. Ingber, and E. Mazur, Mech. Chem. Biosyst. **2**, 17 (2005).
[8] V. Kohli, A. Y. Elezzabi, and J. P. Acker, Lasers Surg. Med. **37**, 227 (2005).
[9] A. Ashkin, J. M. Dziedzic, and T. Yamane, Nature (London) **330**, 769 (1987).
[10] A. Ashkin and J. M. Dziedzic, Proc. Natl. Acad. Sci. U.S.A. **86**, 7914 (1989).
[11] S. M. Block, L. S. B. Goldstein, and B. J. Schnapp, Nature (London) **348**, 348 (1990).
[12] M. Zahn and S. Seeger, Cell. Mol. Biol. (Paris) **44**, 747 (1998).
[13] T. N. Buican, M. J. Smyth, H. A. Crissman, G. C. Salzman, C. C. Stewart, and J. C. Martin, Appl. Opt. **26**, 5311 (1987).
[14] C. Bustamante, Z. Bryant, and S. B. Smith, Nature (London) **421**, 423 (2003).
[15] G. D. M. Jeffries, J. S. Edgar, Y. Zhao, J. P. Shelby, C. Fong, and D. T. Chiu, Nano Lett. **7**, 415 (2007).
[16] M. He, J. S. Edgar, G. D. M. Jeffries, R. M. Lorenz, J. P. Shelby, and D. T. Chiu, Anal. Chem. **77**, 1539 (2005).
[17] J. P. Shelby, J. S. Edgar, and D. T. Chiu, Photochem. Photobiol. **81**, 994 (2005).
[18] C. S. Buer, K. T. Gahagan, G. A. Swartzlander, Jr., and P. J. Weathers, Biotechnol. Bioeng. **60**, 348 (1998).
[19] R. W. Steubing, S. Cheng, W. H. Wright, Y. Numajiri, and M. W. Berns, Cytometry **12**, 505 (1991).
[20] See EPAPS Document No. E-RSINAK-79-028810 for supplementary movie files showing trapping and surgery of living yeast cells using a single laser. For more information on EPAPS, see http://www.aip.org/pubservs/epaps.html.
[21] L. Sacconi, I. M. Tolic-Nørrelykke, R. Antolini, and F. S. Pavone, J. Biomed. Opt. **10**, 014002 (2005).
[22] A. Vogel, J. Noack, G. Hüttman, and G. Paltauf, Appl. Phys. B: Lasers Opt. **81**, 1015 (2005).
[23] K. König, P. T. C. So, W. W. Mantulin, and E. Gratton, Opt. Lett. **22**, 135 (1997).
[24] M. Feuermann, S. Francisci, T. Rinaldi, C. D. Luca, H. Rohou, L. Frontali, and M. Bolotin-Fukuhara, EMBO Rep. **4**, 53 (2003).