Optical micromanipulations inside yeast cells

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We present a combination of nonlinear microscopy and optical trapping applied to three-dimensional imaging and manipulation of intracellular structures in living cells. We use Titanium-sapphire laser pulses for nonlinear microscopy of the nuclear envelope and the microtubules marked with green fluorescent protein in fission yeast. The same laser source is also used to trap small lipid granules naturally present in the cell. The trapped granule is used as a handle to exert a pushing force on the cell nucleus. The granule is moved in a raster-scanning fashion to cover the area of the nucleus and hence displace the nucleus away from its normal position in the center of the cell. Such indirect manipulations of an organelle (e.g., nucleus) can be useful when direct trapping of the chosen organelle is disadvantageous or inefficient. We show that nonlinear microscopy and optical manipulation can be performed without substantial damage or heating of the cell. We present this method as an important tool in cell biology for manipulation of specific structures, as an alternative to genetic and biochemical methods. This technique can be applied to several fundamental problems in cell biology, including the mechanism of nuclear positioning and the spatial coordination of nuclear and cell division. © 2005 Optical Society of America

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1. Introduction

In the last few years there has been a growing interest toward the use of nonlinear optical techniques for the imaging of biological samples. With respect to conventional fluorescence, these techniques offer several advantages, including higher spatial resolution, reduced photodamage of the living sample, reduced photobleaching of the dye, and increased penetration depth. For example, nonlinear microscopy is well suited for high-resolution imaging of intrinsic molecular signals in living cells and tissues, cell motility,

and the distribution of a neurotransmitter in living cells.

On the other hand, optical manipulation techniques have offered new opportunities for applied research in biophysics and nanotechnology. Many of the most powerful optical manipulation techniques are derived from single-beam optical traps known as optical tweezers. Optical tweezers have been used for the manipulation of viruses and cells, as well as for displacement of intracellular organelles. Recently, Ketelaar et al. used optical tweezers to trap and stop the movement of the nucleus of root cells, in order to study how cell growth depends on the location of the nucleus. Single molecules such as DNA and motor proteins have been also studied by use of optical tweezers.

Here we report an integration of optical tweezers with nonlinear microscopy. We show that it is possible to manipulate intracellular structure inside fission yeast cells, and we analyze the trapping of lipid granules in the cytoplasm. We show that trapping does not induce cell death or heating of the cell. Finally, we describe an optical manipulation procedure designed to displace the cell nucleus away from its normal position in the center of the cell. This technique can be used to study the mechanism of nuclear positioning, as well as the spatial coordination of nuclear events (chromosome segregation) and cortical activities (cytokinesis) during cell division.
2. Materials and Methods

A. Cell Preparations

The *Schizosaccharomyces pombe* strain SP837 (*h^90 leu-32 ura4-D18 ade6-216*) was transformed with the green fluorescent protein (GFP)–fusion construct D81717 to visualize the nuclear envelope. The cells were cultured at 30 °C on a synthetic drop-out medium lacking leucine (AA-leu). Visualizing the mitotic spindle was possible with the above strain being transformed with the GFP-/H9251 2-tubulin fusion construct pDQ10518 and cultured at 30 °C on the AA-leu medium plus 2/M thiamine. Before microscopy, the cells were resuspended in liquid AA medium and mounted on a coverslip covered with lectin BS-1 (2 mg/ml, Sigma). Experiments were performed at room temperature (21 °C–22 °C).

B. Experimental Setup

A scheme of the experimental setup is shown in Fig. 1. The expanded beam of a mode-locked Titanium-sapphire laser (Mira 900F, Coherent, 100-fs pulse duration, 80-MHz repetition rate) passes through the scanning system. This system is made of two galvo-mirrors (VM500, GSI Lumunics) and a telescopic lens pair (L1 and L2) that pivot the laser beam from the first galvomirror (GX) to the second one (GY). L3 and TL (see Fig. 1) pivot the laser beam in the back focal plane of the microscope objective (Plan Apo 60X/1.40 Oil, Nikon). The highly focalized laser beam can be used for nonlinear microscopy with the laser in the mode lock (ML) configuration, or it can be used as optical tweezers with the laser in a continuous-wave (cw) configuration. In the nonlinear microscope configuration, the optical sectioning (Z direction) of the sample is achieved with a piezoelectric translator (P-721, Physik Intrumente). The two-photon emission of the specimen is extracted from the backscattered signal by a dichroic mirror (DM), and the signal is filtered by an IR blocking filter (BF), focalized, and collected by an avalanche photon diode (APD). In the optical tweezers configuration, obtaining a strong trap requires that the laser beam be aligned with the optical axis of the objective by use of galvomirrors. In this case we use a XY piezoelectric translator (P-500, Physik Intrumente) to move the sample with respect to the optical trap. Galvomirrors and the XYZ piezo-electric translator are computer-controlled during microscopy and optical manipulation experiments by a software written in LabVIEW (National Instruments).

A typical experiment was performed as follows. First, a three-dimensional image of the cell was taken by two-photon microscopy. Then, the laser parameters were changed from the imaging configuration (ML mode, λ = 880 nm, power in the sample ~1 mW) to the trapping configuration (cw mode, λ = 830 nm, power in the sample ~200 mW). After the optical manipulation, the laser parameters were changed back to those for microscopy and a time-lapse sequence of three-dimensional images was acquired to visualize the effect of the manipulation and the subsequent behavior of the cell.

3. Results and Discussion

A. Trapping of Lipid Granules

Using the experimental setup described above, we could easily trap small lipid granules naturally present in the cell (Fig. 2). We set the laser in cw mode to avoid nonlinear absorption of the GFP in the cell. We chose to use a laser wavelength of 830 nm to reduce photodamage, and a laser power of ~200 mW in the sample to obtain a sufficient force for trapping and moving organelles inside the cytoplasm. The trap location was fixed, while the sample (cells immobilized on a glass coverslip) were moved with the piezoelectric stage. Thus, the cell was moved with respect to the trapped object, but we will refer to this process simply as moving an object through the cell. After establishing the trapping of lipid granules, the next step was to analyze physical aspects of trapping and its influence on cell behavior.
1. Maximum Trapping Force

When a particle is moved at a high velocity, the drag force between the particle and its surroundings is higher than the force exerted by the optical tweezers, hence the particle escapes from the trap.\textsuperscript{22} The maximum force exerted by optical tweezers on a lipid granule was estimated as the drag force at the maximum velocity (\(v_{\text{max}}\)) with which a trapped granule could be moved without escaping from the trap. The granule was moved at velocities from 10 to 30 \(\mu\text{m/s}\), and the escape velocity was found to be greater than 25 \(\mu\text{m/s}\). The apparent viscosity of the cytoplasm was previously measured to be \(\eta = 0.1-0.8\) Pa s.\textsuperscript{23,24} The radius of the granules (\(r\)) was estimated to be 150 nm from electron micrographs of fission yeast cells.\textsuperscript{25} We estimated the force assuming Stoke’s law, \(F = 6\pi\eta v_{\text{max}}r = 10-60\) pN. This value has to be taken with reservation because (i) the cytoplasm is viscoelastic and not purely viscous, though the viscous response dominates at time scales of 0.1 ms to 10 s,\textsuperscript{26} (ii) the Stoke’s law assumption of an infinite medium does not hold in a \(-4 \times 12\) \(\mu\text{m}\) cell surrounded by a stiff wall, and the cell wall proximity implies that the real magnitude of the force is higher than the estimated value, and (iii) the cytoplasm is inhomogeneous and the escape force may be affected by the presence of internal membranes and organelles.

2. Cell Viability

To test for potential photodamage to the cell, we trapped a lipid granule in the cell for 5 min and observed the subsequent behavior of the cell. Trapping did not induce any visible damage to the cell. Yet, when the granules were trapped at a wavelength of 880 nm (used in two-photon microscopy) instead of 830 nm as usual, we observed photodamage effects (a bubble forming near the trapped granule) after a few minutes of trapping. This observation is consistent with the finding of photodamage minimum at 830 nm\textsuperscript{21} in \textit{Escherichia coli}, suggesting that photodamage may be caused by a similar mechanism in different cell types. A minor photodamage can be expected even when a wavelength of 830 nm is used.

We found that the cells were alive for 15 h after trapping, by observing the characteristic motion of the granules similar to that in intact cells (>95% of more than 50 cells),\textsuperscript{26} and by observing that the cells continued growing and dividing. However, the cells with trapped granules grew and divided with a delay of 3–7 h with respect to intact cells (\(n = 4\) cells, Fig. 3A), which indicates that trapping generated stress on the cells. The mechanism of laser-induced damage can be local heating, photochemical processes, or both, creating reactive chemical species. Inasmuch as we did not detect substantial heating of the cell (see Subsection 3.A.3), we assume that the observed delay in cell growth is due to stress caused by the creation of reactive chemical species, e.g., singlet oxygen.\textsuperscript{21}

We propose biological applications of trapping to study microtubule-based processes such as positioning of the nucleus by microtubule forces, as described

**Fig. 3.** (A) Cells are alive, growing, and dividing after laser trapping. Two sister cells were chosen (inset); a granule was laser trapped for 5 min in the cell marked L+ whereas the cell marked L− was intact. Cell length is shown as a function of time. Both cells grew and divided (marked with an asterisk), but the cell with trapped granules showed a delay of \(-7\) h with respect to the intact cell. This delay indicates a moderate laser-induced stress, which the cell can manage. (B) Microtubules are not significantly affected by trapping. A three-dimensional rendering of the interphase microtubule array before (1) and (2) after laser trapping. (C) Trapping does not perturb progression through mitosis. Selected images of a cell in anaphase B without laser trapping (L−) and with trapping (L+). The images marked 1 were taken in midanaphase B, whereas the images marked 2 were taken 7 min later, at the end of anaphase B. In the L+ cell, a granule was trapped close to the cell center between the acquisition of the two images for 5 min. Note that the behavior of the two spindles is very similar. (D) and (E) Trapping does not induce a detectable heating of the cell. (D) Spindle elongation rate (mean ± s.t.d.) in cells in which a granule was trapped for 5 min was not significantly different from that in cells without laser trapping. (E) Spindle elongation rate as a function of temperature. Measurements (circles) are taken from Refs. 28 (data at 20 °C and 36 °C) and 29 (data at 24.5 °C). The shaded area shows our measurements of the spindle elongation rate in cells with laser trapping at room temperature (horizontal lines, mean ± s.t.d.) and the corresponding temperature range (vertical lines). These measurements were performed at room temperature (21 °C–22 °C) and correspond to a range of 17.5 °C–21.5 °C on the graph, suggesting that the cell was not substantially heated by the laser trap. Scale bar in (B) and (C), 2 \(\mu\text{m}\).
in Subsection 3.B. We therefore tested whether trapping affects microtubules. When the trapping was performed in interphase cells, a typical interphase array of microtubules was visible after trapping ($n = 3$ cells, Fig. 3B). The microtubules showed normal dynamics of growth and shrinking. When the trapping was performed in cells that were dividing, the mitotic spindle continued elongating at a normal rate ($n = 10$ cells, Figs. 3C and 3D) and the process of cell division finished as usual. We conclude that trapping does not affect the dynamics of interphase microtubules and of the mitotic spindle, and hence it can be used to study microtubule-related processes, including those during cell division.

3. Laser-Induced Heating in the Cell

In optical trap experiments an intense laser beam is tightly focused at high intensities, which can induce heating of the sample.\(^\text{27}\) The temperature increase in the focus can reach 50 K/W, depending on the properties of the trapped particle and its surroundings.\(^\text{27}\)

For the application of optical tweezers in the study of cell division, as in the current research, it is essential to estimate the overall laser-induced heating of the cell, instead of the heating in the focus. The overall heating can affect the dynamics of cell division activities, whereas the extent of heating of the trapped particle is of secondary importance, since this particle is used merely as a handle, as described (see Subsection 3.B). We estimated the overall heating in the cell by investigating a well-characterized temperature-dependent process: the elongation of the mitotic spindle in Anaphase B.\(^\text{28}\)

To examine whether trapping increases the spindle elongation rate, we used cells with a mitotic spindle marked by GFP (Fig. 3C). We chose those in midanaphase B (using the imaging configuration of the setup), and trapped a granule close to the cell center for 5 min (using the trapping configuration). The 5-min interval was chosen because the application of this method (the displacement of the nucleus) required trapping of the granule for up to 5 min (see Subsection 3.B). The measured spindle elongation rate during the period of optical trapping in the cell was $0.35 \pm 0.14 \mu m/min$ (mean ± s.t.d., $n = 10$ cells). As a control, the spindle elongation rate in intact cells at room temperature was measured to be $0.36 \pm 0.11 \mu m/min$ ($n = 14$).

The two groups of elongation rates (with and without trapping) were not significantly different ($t$-test, $p = 0.81$, Fig. 3D). The previously published spindle elongation rates are $0.38 \pm 0.1 \mu m/min$ at 20 °C,\(^\text{26}\) $0.67 \pm 0.18 \mu m/min$ at 24 °C–25 °C,\(^\text{25}\) and $1.4 \pm 0.2 \mu m/min$ at 36 °C.\(^\text{26}\)

The rates measured here in the cells with laser trapping correspond to a temperature range of 17.5 °C–21.5 °C, considering elongation rate as a function of temperature (Fig. 3E). These results indicate that the cell was not substantially heated by the laser trap.

B. Optically Induced Displacement of the Cell Nucleus

In fission yeast, the nucleus is positioned in the center of the cell. The site of cell division, which also coincides with the cell center, seems determined by the position of the predivision nucleus.\(^\text{30}\) To study the mechanism of nuclear centering and the coordination between the location of the nucleus and of the division plane, we set out to displace the cell nucleus away from the cell center. Using the experimental setup described in Subsection 2.B, it was not possible to optically trap the yeast nucleus, probably because of an insufficient difference between the refractive indices of the nucleus and the surrounding cytoplasm. Thus we trapped lipid granules in the cytoplasm, also as described in Subsection 3.A, and used them as handles to push the nucleus.

We tried to displace the nucleus along the long cell axis ($x$ axis) by trapping a granule close to one side of the nucleus and moving it toward the nucleus along the $x$ axis. This procedure, however, rarely resulted in a detectable displacement of the nucleus. The nucleus is soft and round, thus the trapped granule can deform the nucleus and pass without displacing it, especially if the starting position of the granule does not coincide with the center of the nucleus along the $y$ axis (the short axis of the cell) and the $z$ axis. Therefore, we designed a scanning-trap technique in which the trapped granule is being moved along a raster-scanning trajectory over a $2 \mu m \times 2 \mu m$ area in the $y$–$z$ plane, covering the projection of the nucleus on the $y$–$z$ plane. The trap moves simultaneously in the $x$ direction, as shown in Fig. 4. The speed of the trap movement was $25 \, nm/s$ in the $x$ direction, $2.5 \, \mu m/s$ in the $y$, and $0.125 \, \mu m/s$ in the $z$. We chose this set of values as a compromise between a high efficiency of nuclear displacement, which requires slow movements, and a short laser-irradiation time of the cell.

To measure the nuclear position, we used cells in which the nuclear envelope was marked by GFP. After acquiring a two-photon image of the cell, we applied the scanning-trap technique for 3–5 min. An example of a cell with the displaced nucleus is shown.
in Fig. 5A. Optical sections along the z axis (Fig. 5B) indicate that the scanning-trap technique displaced the whole nucleus, instead of deforming it without a net displacement. The maximum displacement obtained in a group of more than 20 cells was 2 μm, whereas movements of the center of nonmanipulated nucleus in interphase do not exceed 0.5 μm.

The nuclear envelope consists of an inner and an outer nuclear membrane, connected through nuclear pores. The outer membrane is continuous with the endoplasmatic reticulum (ER). Manipulation of the nucleus most likely results in remodeling of this network of membranes. If the ER is attached to the cell cortex in the vicinity of the nucleus, a larger force may be needed to displace the nucleus. This kind of attachment may be the reason the optical manipulation did not result in a detectable displacement of the nucleus in ~30% trials.

After the nucleus had been displaced by the optical trap, it spontaneously moved back toward its original position at the cell center (Fig. 6). What is the mechanism that positions the nucleus in the cell center? One can directly test models of nuclear positioning, which typically involve microtubule-dependent forces, by optically displacing the nucleus and studying its return toward the cell center using microscopy under native or nonnative (drug treatments and/or mutant cell) conditions. Our observation that trapping does not affect microtubule dynamics supports the use of optical trapping for studies of nuclear positioning. Furthermore, the central position of the yeast nucleus coincides with the position of the cell division plane. Coordination of the nuclear position and the site of cell division is crucial for cell survival: After separation of the divided nuclei, the cell has to divide between them, for successful distribution of the genetic material to the daughter cells to occur. Coordination of the nuclear and the division plane position can be probed directly by optical manipulations of the nuclear position at various stages of the cell cycle. Optical trapping does not affect the dynamics of cell division and allows for observation of a single cell both before and after the manipulation, hence it is possible to know the exact stage of the cell cycle at which the manipulation is performed. The latter may be an important advantage of the trapping method over more commonly used centrifugation, in studies in which timing is crucial during displacement of cell organelles.

4. Conclusions
Optical micromanipulations of cell organelles can be performed even when the chosen organelle cannot or should not be trapped directly, by trapping another intracellular structure and using it as a handle to exert force on the chosen organelle. This technique does not induce gross damage or heating of the cell.
After trapping, the cell continues growing and divides, though with a delay. This indicates that the laser induces stress on the cell, to an extent the cell can manage. If trapping is performed during cell division, the mitotic spindle elongates at a normal rate and the division completes as usual, suggesting that the dynamics of cell division is not affected by trapping; hence trapping can be used to study division-related processes. Optical manipulations, combined with two-photon microscopy and laser nanosurgery, can be used to directly test models of cell mechanics involving forces and spatial order in a living cell. Outstanding problems in cell biology, such as positioning of cell organelles, centering of the mitotic spindle, and spatial coordination of nuclear and cell division, can be addressed by these methods.

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References
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