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Growth of Budding Yeasts under Optical Trap

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Abstract

Optic tweezer is powerful tool to investigate biologic cells. Of eukaryotic cells, it was poorly documented regarding to optic trapping to manipulate yeasts. In preliminary experiment to explore yeast biology, interferometric optical tweezers was exploited to trap and manipulate budding yeasts. Successfully, several budding yeasts are trapped simultaneously. We found that the budding direction of the daughter cell was almost outward and the daughter cell surrounded by other yeasts grows slowly or fail to grow. Thus it was assumed that neighboring cells around budding yeast may be critical in budding and the growth of daughter cells. This is first report pertaining to the pattern of yeast budding under the optical trap when multiple yeasts were trapped.

Keywords: Optical trap, Interferometric optical tweezers, Budding yeast cell

Since Ashkin *et al*. demonstrated the optical tweezers is used to trap and manipulate microparticles including biological cells[1], the optical tweezers become a powerful tool in biological sciences. The optical tweezers can be used to trap and manipulate three dimensionally micron-sized particles in both aqueous media and air. The optical trapping force is induced by the transfer of momentum of incident photons to the particles[2] and is proportional to the gradient of light intensity. For a highly focused beam, the particles were moved and trapped to near the focal point because of the gradient of focused laser beam intensity. The optical trapping force induced by optical tweezers is pico-Newton level forces enough to trap or manipulate the particles including biological cells. This optical trapping technique offer researchers unprecedented experiments at the single cell or the single molecule level.

The optical tweezers incorporated with position detection techniques[3,4] have been used to measure the physical or mechanical properties of biological cell such as force, motion[5-7], viscosity, and elasticity[8-10]. The interference of multiple beams[11-13], the diffractive optical elements[14], or the spatial light modulator[15] is used to trap multiple particles.

In this paper, we attempted to employ an interferometric optical tweezers to trap simultaneously multiple microparticles including biological cells. This is first report pertaining to the pattern of yeast budding under the optical trap when multiple yeasts were trapped.

This study showed that optical tweezers are a powerful tool for yeast cell biology research. Prior to live cell trapping, we attempted to establish the optimal condition of optical tweezer to test. Figure 1 shows the schematic of an interferometric optical tweezers. We used a laser diode (λ=834 nm, max. power=200 mW) as a light source. The linearly polarized laser beam was divided by a beam splitter. Two laser beams reflected by two mirrors were incident into an objective lens (40×, NA=0.6) in a conventional optical microscope. An interference pattern was generated in the specimen plane. The particles were the polystyrene spheres and the yeast, which were suspended in the distilled water and the culture medium, respectively. The chamber was made up of the slide glass and the cover glass. The inset in Figure 1 shows the multiple polystyrene spheres (diameter= 5 μm) trapped in the interference pattern. We can trap and manipulate simultaneously multiple particles laterally in the specimen plane. Next, for live cell trapping, we chosed *Saccharomyces cerevisiae* (strain SK) as prototypic model of budding yeast. The interferometric optical tweezers was applied to trap and manipulate single yeast cells in the specimen plane. In order to identify whether the focused laser beam gives any damages to the trapped budding yeast, we monitored the trapped budding yeast for over one hour. As shown in Figure 2, the trapped yeast budded in the chamber. Sequential frames show that there is no optical damage occurred on the budding yeast for

λ=834 nm. It was reported that the photodamage exhibits minima at 830 nm in the case that the single cells of *Escherichia coli* were trapped[16]. As the light intensity was incident on the budding yeast, the daughter cell started to move to the center and the shape of the mother cell became circular. This is because the laser beam is circular and the gradient of laser beam intensity is the largest at the center of the interference pattern. We also compared the size of the trapped daughter cell with that of the daughter cell out of the trap for the same hour. There is no difference between two daughter cells.

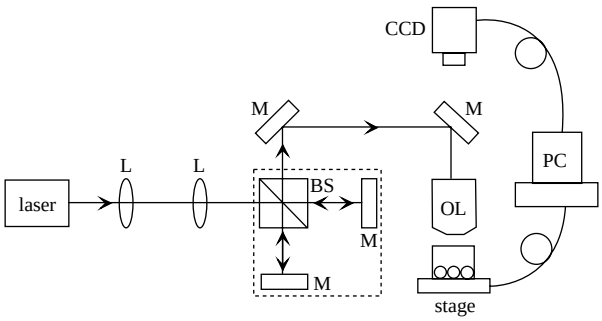

Figure 1. Schematic of an interferometric optical tweezers. L: lens, M: mirror, BS: beam splitter, OL: objective lens, stage: motorized translation stage. The dashed box indicates the Michelson interferometer. The inset figure shows the multiple polystyrene spheres (diameter=5 μm) trapped in the interference pattern.

Figure 3 shows several budding yeasts simultaneously trapped by the interferometric optical tweezers. It was shown that the trapped yeasts budded without any damages. We trapped a budding yeast cell and moved near two budding yeasts cells, as shown in the sequential frame of Figure 3(a). After we trapped simultaneously three budding yeast cells to make one of the daughter cell surrounded by other cells, we observed how the daughter cell in the dashed circle grows further. As shown in Figure 3(a), the daughter cell grows slowly, compared with not-surrounded daughter cells. Another sequential frame of Figure 3(b) shows that the three yeast cells which didn't seem to start budding were trapped simultaneously.

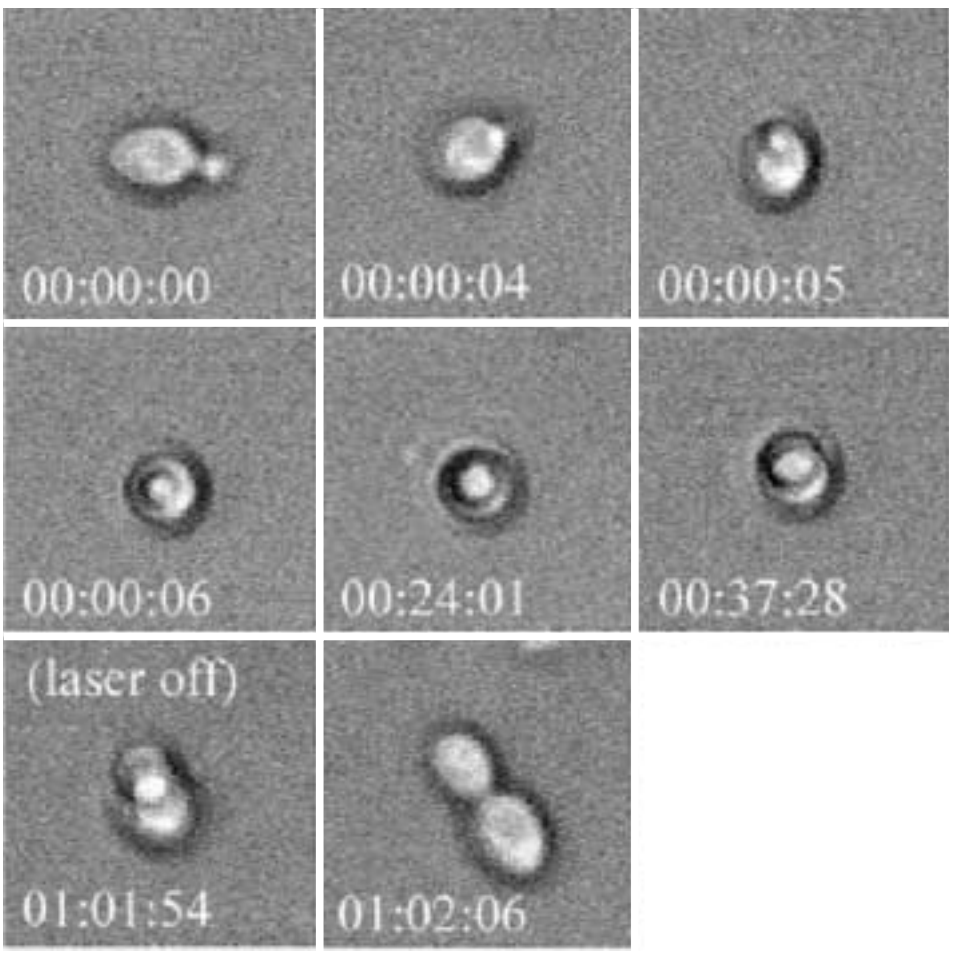

Figure 2. Sequential frames of the budding yeast cell in the optical trap. The figures show that there is no optical damage occurred on the budding yeast for λ=834 nm. After the laser was off, it was confirmed that the daughter cell grew without damages.

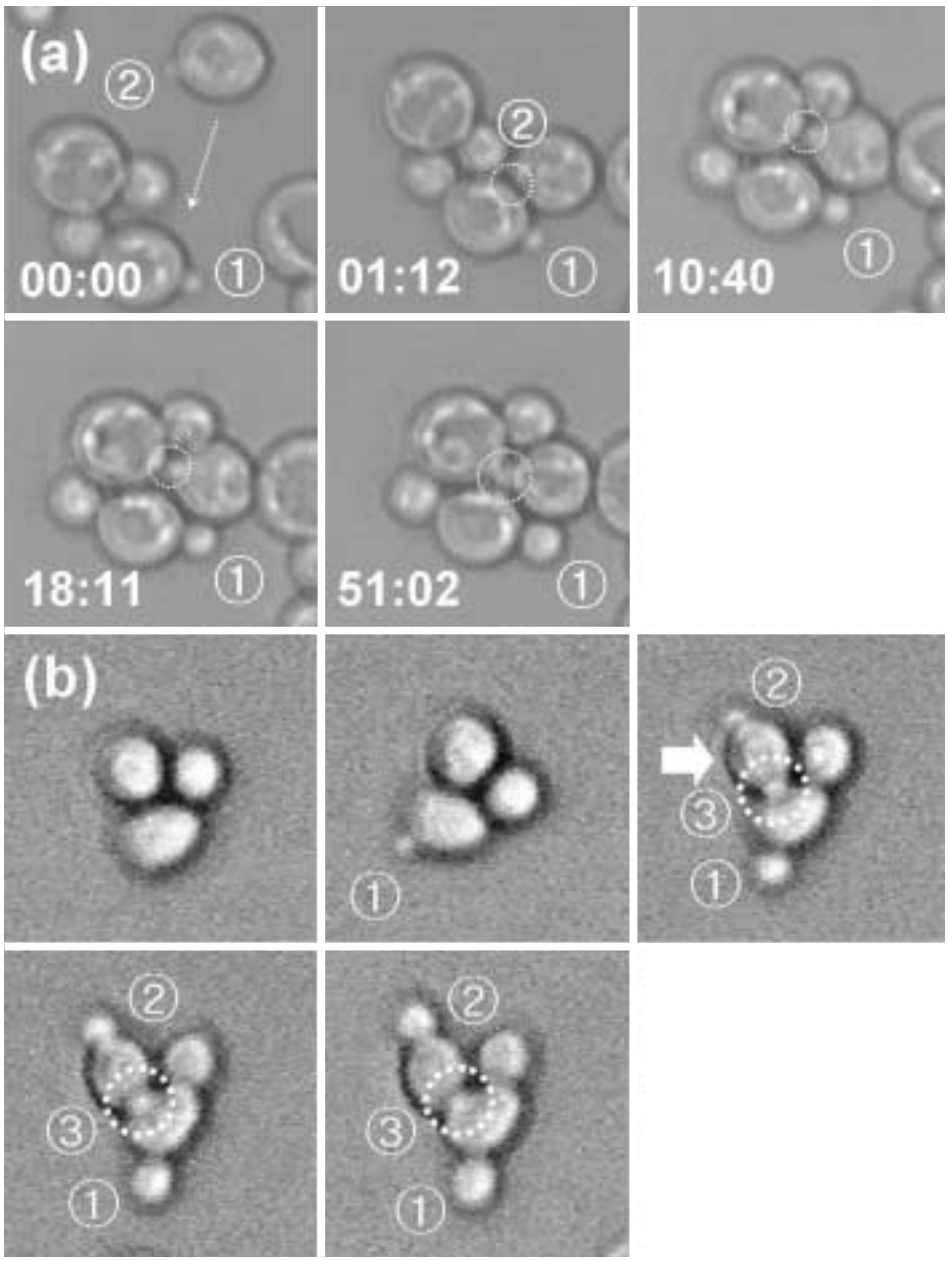

Figure 3. Sequential frames of three budding yeast cells in the optical trap. (a) The daughter cell was forced to be surrounded by other cells. The daughter cell surrounded by other cells didn't grow as fast as another daughter cell. (b) One mother cell pointed by a thick arrow had two daughter cells. The daughter cell that grew downward near the neighboring cells didn't grow any more.

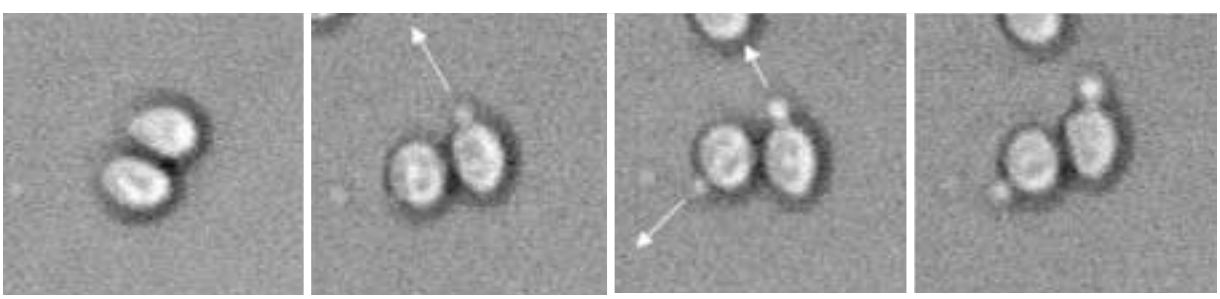

Figure 4. Sequential frames of two budding yeast cells in the optical trap. The directions of budding were outward. The elapsed time was about 1 hour.

As time went by, the trapped yeast cells started budding in the optical trap. One of the trapped budding yeast cells pointed by a thick arrow had two daughter cells. One of the daughter cells (2) grew upward, and the other (3) in the dashed circle grew downward near other yeast cells. Compared (3) with (1), we knew that (3) grew slowly. After the laser was off, the daughter cell didn't grow further. We suggest that the neighboring cells may be critical in budding and the growth of daughter cells. Figure 4 shows that two yeast cells in resting stage were trapped simultaneously. As time went by, the trapped yeast cells started budding. As shown in the figure, the directions of budding were outward. We repeated the same process several times. The results were the same.

Discussion

In summary, we used an interferometric optical tweezers to trap and manipulate simultaneously multiple particles. Successfully, several budding yeasts are trapped simultaneously. We found that the budding direction of the daughter cell was almost outward and the daughter cell surrounded by other yeasts grows slowly or fail to grow. Thus it was assumed that neighboring cells around budding yeast may be critcal in budding and the growth of daughter cells. This is first report pertaining to the pattern of yeast budding under the optical trap when multiple yeasts were trapped.

Such an optical tweezers combined with live-cell imaging will confer great insight into understanding eukaryotic cell biology.

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