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REVIEW

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Experimentally manipulating fungi with optical tweezers

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Abstract A short review of the use of optical tweezers in fungal cell biological research is provided. First, we describe how optical tweezers work. Second, we review how they have been used in various experimental live-cell studies to manipulate intracellular organelles, hyphal growth and branching, and whole cells. Third, we indicate how optically trapped microbeads can be used for the localized delivery of chemicals or mechanical stimulation to cells, as well as permitting measurements of the growth forces generated by germ tubes. Finally, the effects of optical trapping on fungal cell viability and growth are assessed.

Key words Germ tubes · Hyphal tip growth · *Neurospora crassa* · Optical tweezers · Spitzenkörper

Introduction

Optical tweezers (also termed laser tweezers or optical traps) allow microscopic particles, living cells, and organelles to be trapped and manipulated with light. Since their invention 20 years ago (Ashkin et al. 1986), optical tweezers have been employed in a wide variety of biological research applications (Block 1990; Kuo and Sheetz 1992; Ashkin 1997; Sheetz 1998; Greulich 1999; Molloy and Padgett 2002; Neuman and Block 2006). So far, however, optical tweezers have been little used as experimental tools in fungal cell biology (Wright et al. 2007). In this short review we provide a summary of work in this area.

How optical tweezers work

Optical tweezers facilitate the noninvasive micromanipulation of both inert and biological microscopic particles solely by using light. They utilize an intense laser light source, which is tightly focused by a microscope objective lens of high numerical aperture to produce a three-dimensional optical trap. When photons are absorbed, reflected, or refracted by a transparent object, the momentum they possess is changed, which corresponds to an action force acting on these photons. Newton's third law states that for every action force there is a corresponding reaction force that is equal in magnitude and opposite in direction; therefore, the object creating the action force will have the reaction force applied to it (Fig. 1). With a laser beam of Gaussian profile, the net force experienced by a spherical object is zero at a point close to the focus of the laser beam. This point is then the center of the optical trap. When the object is displaced slightly from this trap center, it experiences a restoring force that is proportional to the displacement, up to a certain maximum, after which the object escapes. The trapping forces generated are typically of the order of piconewtons and sufficient to trap and move microscopic objects up to tens of micrometers in size. For an object to be trapped efficiently, it must have a refractive index that is sufficiently higher than its surroundings (Fig. 2). Tweezer systems can be equipped with multiple traps either by using more than one laser or by generating holographic optical tweezers with a computer-controlled spatial light modulator from a single laser source (Grier and Roichman 2006).

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Methodology

We have built a simple, compact, easy-to-use, safe, and robust dual-trap optical tweezer system that can be used with brightfield, phase-contrast, differential interference contrast, and fluorescence optics. This apparatus has been mounted on both Nikon TE2000U and Olympus IX70 inverted light microscopes and is an upgraded version of the one-trap tweezer system that we have described previously (Wright et al. 2007). In our latest tweezer system, the location of one trap in the field of view is computer-controlled using galvanometric mirrors, allowing easy and accurate positioning of trapped objects with the click of a mouse (Wright et al. 2007). The other trap is fixed in the center of the field of view and requires the microscope stage to be moved to change the position of a trapped object. Both lasers are 785-nm diode lasers with a maximum output power of 70 mW.

Our experimental systems are conidia, conidial germlings, and vegetative hyphae of *Neurospora crassa*. Filamentous fungi are well suited to being micromanipulated by optical tweezers because they can be analyzed as a monolayer of cells or hyphae, which are sometimes very large, and can exhibit very rapid growth responses to physical and chemical stimuli (Wright et al. 2007).

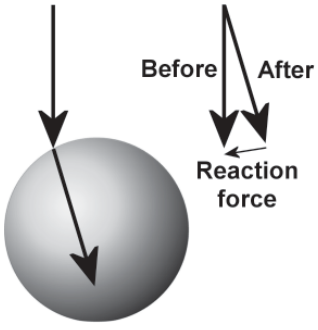

Fig. 1. A ray diagram depicting the interaction of a single ray of light with a transparent sphere. The sphere refracts the light changing its direction and therefore momentum (an action force). The resultant reaction force is opposite in direction and equal in magnitude (Newton's third law). By focusing a laser beam of Gaussian profile through a microscope objective lens of high numerical aperture, the net reaction force is to trap the object at the region of highest light intensity, the point of focus

Manipulating organelles of high and low refractive index

Organelles that are of higher refractive index than their surrounding cytoplasm can be trapped with optical tweezers. Good examples of this are the highly refractile Woronin bodies that are involved in septal pore occlusion (Berns et al. 1992; Wright et al. 2007; Wright et al. 2007: supplementary movie 3: www.fungalcell.org/tweezermovies.htm). Once trapped, organelles can usually be easily moved as long as they are not strongly tethered in place (e.g., to cytoskeletal elements). Figure 2 shows the trapping and movement of a spherical organelle across a hypha. The cytoplasm is a highly viscous environment, and as a result moving an organelle must be done slowly to prevent it being lost from the trap. In contrast, organelles of lower refractive index, such as vacuoles, are repelled from the trap (Wright et al. 2007). Although this makes it more difficult, tweezers can still be used to micromanipulate organelles by a technique that involves "chasing" them in a manner akin to repelling magnets (Wright et al. 2007: supplementarys movies 4 and 5: www.fungalcell.org/tweezermovies.htm). It is also possible to press repelled vacuoles against the plasma membrane and split them in two (Wright et al. 2007; Wright et al. 2007: supplementary movie 6: www.fungalcell.org/tweezermovies.htm).

Manipulating hyphal growth and branching

The Spitzenkörper, the secretory vesicle-dominated organelle complex that regulates tip-growth (Bartnicki-Garcia 2002; Harris et al. 2005; Virag and Harris 2006), was previously shown to be unexpectedly repelled by optical trapping (Bracker et al. 1997; Wright et al. 2005, 2007). The reason for this dark-phase (high refractive index) structure being repelled rather than trapped is not understood, and more than one mechanism may be involved (Wright et al. 2007). The direction of tip growth can be repeatedly redirected, causing a "zigzag" pattern of hyphal growth (Fig. 3; also see Wright et al. 2007: supplementary movie 7: www.fungalcell.org/tweezermovies.htm). Alternatively, parking the optical trap at one point beneath the hyphal surface can induce branch formation, probably as a result of concentrating secretory vesicles in that location (Bracker et al. 1997).

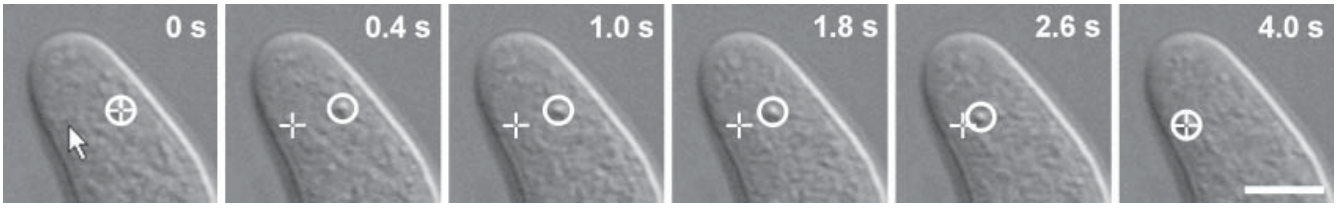

Fig. 2. Differential interference contrast microscopy showing the trapping and moving of a spherical organelle of high refractive index within a hypha of *Neurospora crassa*. The sequence shows the organelle being moved laterally across the hypha. The current position of the trap is represented by the *circle*; the *cross-hair* shows the position to which the trap is being moved. *Bar* 5 μm

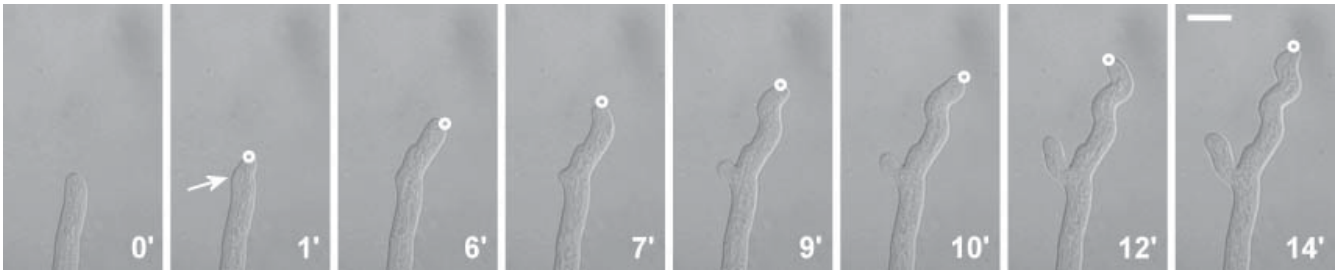

Fig. 3. Differential interference contrast microscopy showing redirection of growth by manipulating the Spitzenkörper. When the laser is positioned adjacent to the Spitzenkörper hyphal tip, growth becomes reoriented away from the trap because the Spitzenkörper is repelled by it. The trap was repeatedly repositioned to repeatedly redirect tip growth, giving rise to the zigzag growth pattern of the hypha. A branch was formed at the point of initial exposure to the trap (*arrow* at *1 min*). The current position of the trap is represented by the *circle*. *Bar* 10 μm

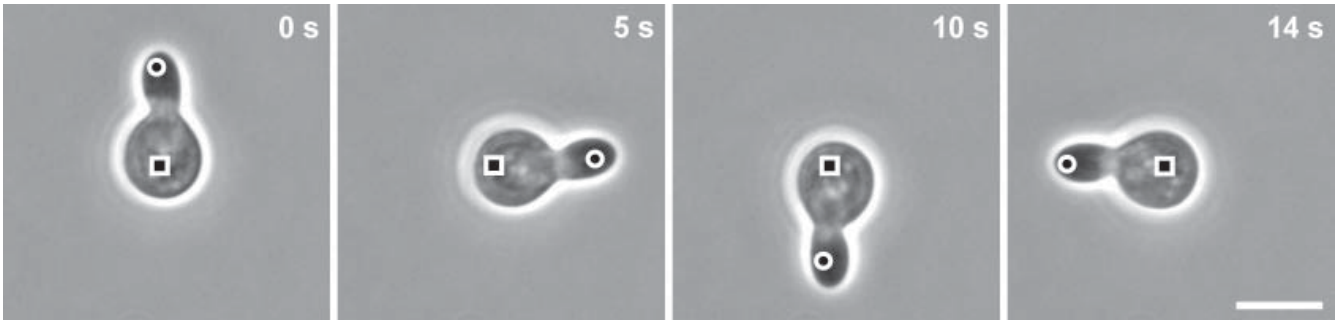

Fig. 4. Phase-contrast microscopy showing rotation of a conidial germling using two traps. The conidium is held by the stationary trap (*square*); the germ tube tip is held by the moveable trap (*circle*). *Bar* 5 μm

Manipulating whole cells to study cell–cell signalling

Whole spores or spore germlings can be trapped and moved around in liquid culture or in a liquid film on solid medium (Wright et al. 2007; Wright et al. 2007: supplementary movies 1 and 2: www.fungalcell.org/tweezermovies.htm). Moving whole spores with optical tweezers has been used to investigate the signaling that occurs between conidial anastomosis tubes (CATs) that are homing towards each other (Fleiβner et al. 2005; Roca et al. 2005). With the recent addition of a second trapping laser into our system, we are now able to trap two germinating spores simultaneously and reposition one with respect to the other; this helps overcome the problem of one of the homing spore germlings drifting away from the other over the typical 20- to 30-min period of the CAT homing assay (Fleiβner et al. 2005). Another use of two traps is to enable precise control over the orientation of germinated spores. In Fig. 4, the conidium was trapped with the stationary laser whilst the emergent germ tube tip was trapped with the steerable laser.

We have developed a technique using optical tweezers to establish arrays of spores that are adhered to coverslips. By coating half the area of a coverslip with polyvinyl alcohol (PVA), a nonstick region was created (Krylov and Dovichi 2000). Ungerminated conidia were then trapped with the optical tweezers and carried from the PVA region to the uncoated area. Briefly holding the conidia against the glass caused them to adhere to it. Repeating this procedure allowed fixed arrays of spores to be generated in which the distance separating cells could be accurately defined (Fig. 5). This method provides another experimental approach to study communication between cells within a cell population (e.g., in relation the signals involved in CAT homing and germ tube avoidance; Roca et al. 2005).

Holographic optical tweezers potentially provide a more elegant way of producing microarrays of trapped cells. Here, multiple traps are generated from a single laser source, and each trap can be individually computer controlled. Lafong et al. (2006) successfully used a holographic tweezer system to trap arrays of up to six cells of *Saccharomyces cerevisiae* and conidia of *N. crassa* and to move the cells relative to one another.

Using optically trapped microbeads as experimental tools

Microbeads that are 1–10 μm in diameter, and that have a higher refractive index than their surroundings, can be efficiently trapped with optical tweezers. Commonly, these microbeads are made of polystyrene or silica and are widely available commercially. Once trapped they can be used as tools to experimentally manipulate biological samples.

Chemicals can be delivered to localized regions of cells with microbeads by (1) soaking porous microbeads in a

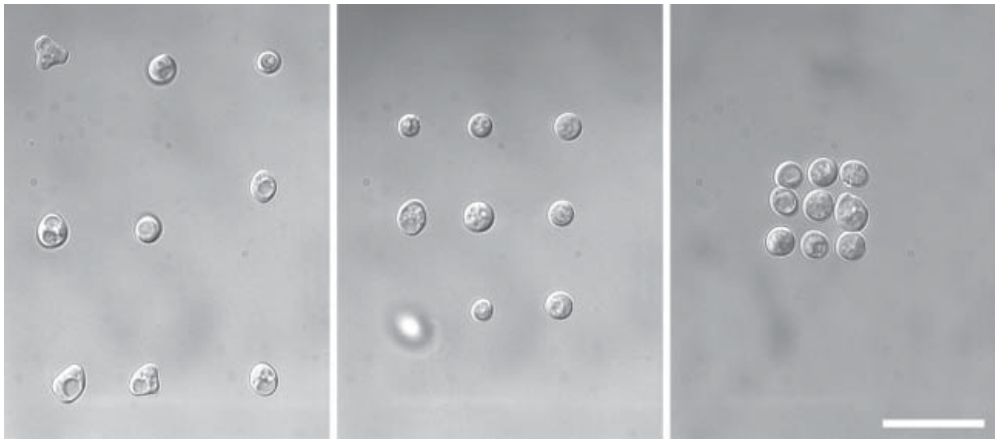

Fig. 5. Differential interference microscopy showing the production of microarrays of conidia. Macroconidia were trapped in an area of coverslip made nonsticky by coating it with polyvinyl alcohol (PVA) and then moved carefully using the microscope stage controls, up to 1 cm across the coverslip, to an uncoated region of the coverslip where they were carefully positioned and brought into contact with the uncoated glass to adhere. These spore arrays were stable and could be observed over long periods of time whilst germination took place. *Bar* 25 µm

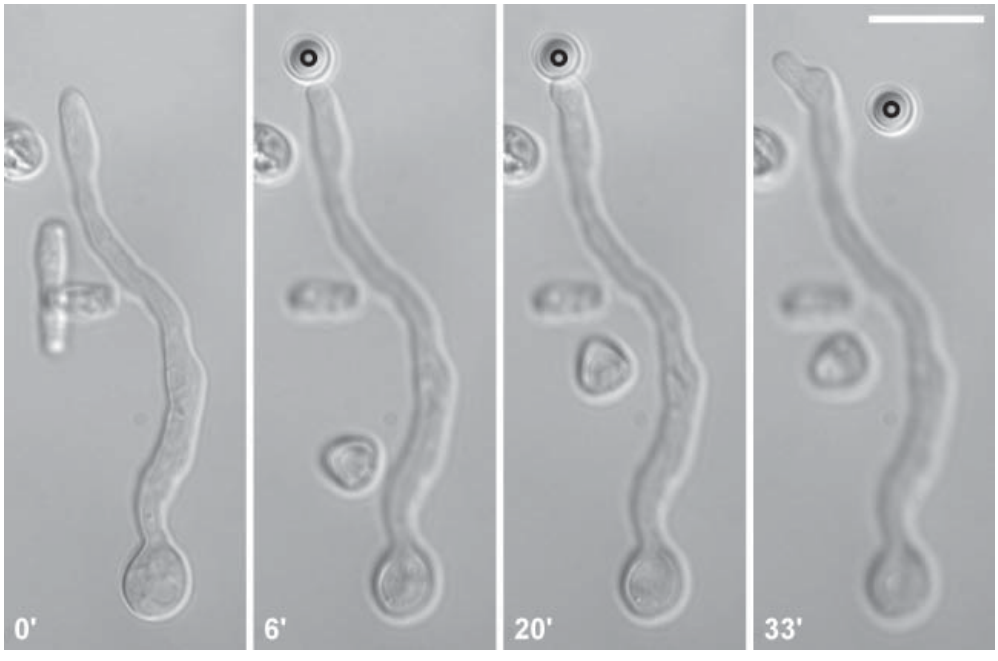

Fig. 6. Differential interference microscopy showing the obstruction of germ tube growth with an optically trapped 4-µm bead. Note that upon first making contact with the bead the germ tube tip moved the bead very slightly but was unable expel it from the trap; this was subsequently followed by swelling of the germ tube and cessation of its growth. When the obstacle was removed, extension of the germ tube resumed. The highest output laser power (70 mW) was used in this experiment, which equates to a trapping force of 19 pN (Wright et al. 2005). *Bar* 10 µm

chemical solution to provide a continuous release of the chemical from the beads (Wright et al. 2007), (2) trapping a chemical in microcapsules and releasing it on demand (Sun and Chiu 2004), or (3) pressing against a cell surface trapped microbeads that have been coated with chemicals (Wei et al. 1999).

A mechanostimulus can be locally applied to cells or hyphae by hitting them with a trapped bead. Wright et al. (2007) demonstrated this using a 10-µm polystyrene microbead to repeatedly hit growing vegetative hyphal tips. The effect of this treatment was to cause a slight redirection of hyphal growth without changing the rate of hyphal extension (Wright et al. 2007: supplementary movie 10: www.fungalcell.org/tweezermovies.htm).

Trapped microbeads can be used to measure growth forces produced by fungal cells. The force required to displace a bead from an optical trap can be calibrated (Wright et al. 2005) and hence optical tweezers can be used to measure forces applied to trapped beads (Wright et al. 2007 supplementary movie 11: www.fungalcell.org/tweezermovies.htm). The maximum trapping force varies with the size of the bead and, for a given size of bead, linearly increases with the laser power. In most cases, the forces produced by leading vegetative hyphal tips are probably far in excess of those which laser tweezers can generate (Money et al. 2004; Wright et al. 2005, 2007; Wright et al. 2007: supplementary movie 8: www.fungalcell.org/tweezermovies.htm). However, we found that a conidial germ tube will push on a trapped bead and move it slightly but not expel it from the trap. If the trap is maintained in position, the germ tube subsequently swells and stops growing (Fig. 6; Wright et al. 2007: supplementary movie 9: www.fungalcell.org/

tweezermovies.htm). These results indicated that germ tubes generate significantly smaller growth forces than leading vegetative hyphae (Wright et al. 2006).

Effects of optical tweezers on cell viability

Optical tweezers are often described as a "non-invasive" technique for studies with living cells. However, significant photodamage can occur if the user chooses a suboptimal combination of laser wavelength, laser power, and duration of exposure to the laser beam, as well as taking into account the type of biological sample being irradiated (Liang et al. 1996; Neuman et al. 1999). Wavelengths that have been successfully used as optical traps in studies on living fungal cells are 647 nm (Lafong et al. 2006), 760 nm (Berns et al. 1992), 785 nm (Fleiβner et al. 2005; Roca et al. 2005; Wright et al. 2005, 2007; also this article), 820 nm (Bracker et al. 1997), and 830 nm (Arneborg et al. 2005; Sacconi et al. 2005). We found that a 30-s exposure of ungerminated conidia of *N. crassa* to a 785-nm trapping laser had no significant effect on the onset of conidial germination, the rate of germination, or the final percentage of conidia that germinated (Wright et al. 2007). Furthermore, conidial germlings continuously optically trapped for 25 min exhibited no deleterious effects with regard to conidial anastomosis tube growth, homing, or fusion (Wright et al. 2007). Yeast cells of *S. cerevisiae* were found not to be substantially heated by trapping them with optically tweezers at 830 nm. Potential heating of these cells was assessed by monitoring spindle pole elongation, which is a temperature-sensitive process (Sacconi et al. 2005).

Conclusions

We have used optical tweezers in a range of fungal cell biology applications to trap and micromanipulate whole fungal cells, organelles, and beads. Optical tweezers are very powerful experimental tools that will have numerous applications in mycological research in the future. They will be particularly important when integrated with the analysis of living cells using advanced imaging techniques.

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References

Arneborg N, Siegumfeldt H, Andersen GH, Nissen P, Daria VR, Rodrigo PJ, Glückstad J (2005) Interactive optical trapping shows that confinement is a determinant of growth in a mixed yeast culture. FEMS Microbiol Lett 245:155–159

Ashkin A (1997) Optical trapping and manipulation of neutral particles using lasers. Proc Natl Acad Sci USA 94:4853–4860

Ashkin A, Dziedzic JM, Bjorkholm JE, Chu S (1986) Observation of a single-beam gradient force optical trap for dielectric particles. Opt Lett 11:288–290

Bartnicki-Garcia S (2002) Hyphal tip growth: outstanding questions. In: Osiewacz HD (ed) Molecular biology of fungal development. Dekker, New York, pp 29–58

Berns MW, Aist JR. Wright WH, Liang H (1992) Optical trapping in animal and fungal cells using a tunable, near-infrared titanium-sapphire laser. Exp Cell Res 198:375–378

Block SM (1990) Optical tweezers: a new tool for biophysics. In: Foster GA, Gross SP (eds) Noninvasive techniques in cell biology. Wiley, New York, pp 375–402

Bracker CE, Murphy DJ, Lopez-Franco R (1997) Laser microbeam manipulation of cell morphogenesis in growing fungal hyphae. Proc SPIE 2983:67–80

Fleiβner A, Sarkar S, Jacobson DJ, Roca MG, Read ND, Glass NL (2005) The *so* locus is required for vegetative cell fusion and postfertilization events in *Neurospora crassa*. Eukaryot Cell 4:920–930

Greulich KO (1999) Micromanipulation by light in biology and medicine: the laser microbeam and optical tweezers. Birkhauser, Basel

Grier DG, Roichman Y (2006) Holographic optical trapping. Appl Opt 45:880–887

Harris SD, Read ND, Roberson RW, Shaw B, Seiler S, Plamann M, Momany C (2005) Polarisome meets Spitzenkörper: microscopy, genetics, and genomics converge. Eukaryot Cell 4:225–229

Krylov SN, Dovichi NJ (2000) Single-cell analysis using capillary electrophoresis: influence of surface support properties on cell injection into the capillary. Electrophoresis 21:767–773

Kuo SC, Sheetz MP (1992) Optical tweezers in cell biology. Trends Cell Biol 2:116–118

Lafong A, Hossack WJ, Arlt J, Nowakowski TJ, Read ND (2006) Time-multiplexed Laguerre-Gaussian holographic optical tweezers for biological applications. Opt Express 14:3065–3072

Liang H, Vu KT, Krishnan P, Trang TC, Shin D, Kime, S, Berns MW (1996) Wavelength dependence of cell cloning efficiency after optical trapping. Biophys J 70:1529–1533

Molloy JE, Padgett MJ (2002) Lights, action: optical tweezers. Contemp Phys 43:241–258

Money NP, Davis CM, Ravishankar JP (2004) Biomechanical evidence for convergent evolution of the invasive growth process among fungi and oomycete water molds. Fungal Genet Biol 41:872–876

Neuman KC, Block SM (2006) Optical trapping. Rev Sci Instrum 75:2787–2809

Neuman KC, Chadd EH, Liou GF, Bergman K, Block SM (1999) Characterization of photodamage to *Escherichia coli* in optical traps. Biophys J 77:2856–2863

Roca MG, Arlt J, Jeffree CE, Read ND (2005) Cell biology of conidial anastomosis tubes in *Neurospora crassa*. Eukaryot Cell 4:911–919

Sacconi L, Tolić-Nørrelykke IM, Stingari C, Antolini R, Pavone S (2005) Optical micromanipulations inside yeast cells. Appl Opt 44:2001–2007

Sheetz MP (1998) Laser tweezers in cell biology. In: Sheetz MP (ed) Methods in Cell Biology, vol. 55, Academic, San Diego, pp xi–xii

Sun B, Chiu DT (2004) Synthesis, loading, and application of individual nanocapsules for probing single-cell signaling. Langmuir 20:4614–4620

Virag A, Harris SD (2006) The Spitzenkörper: a molecular perspective. Mycol Res 110:4–13

Wei X, Tromberg BJ, Cahalan MD (1999) Mapping the sensitivity of T cells with an optical trap: polarity and minimal number of receptors for Ca^{2+} signaling. Proc Natl Acad Sci USA 96:8471–8476

Wright GD, Arlt J, Poon WCK, Read ND (2005) Measuring fungal growth forces with optical tweezers. Proc SPIE 5930:F1–F7

Wright GD, Arlt J, Poon WCK, Read ND (2007) Optical tweezer micromanipulation of filamentous fungi. Fungal Genet Biol (in press)