Manipulating fungi with laser tweezers

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Optical tweezers were first described in the 1980’s and have been implemented in a wide variety of research across many disciplines; however, the attention received from fungal biologists has been limited. In an attempt to correct the balance we have evaluated what can be achieved using optical tweezers to micromanipulate cells of the model filamentous fungus \textit{Neurospora crassa}. Optical tweezers facilitate the non-invasive micromanipulation of both inert and biological microscopic particles simply using light. They utilise an intense laser light source, which is tightly focused by an objective lens of high numerical aperture to produce a 3-dimensional trap at the point of focus. Basically, 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. The trapping forces produced are of the order of piconewtons and are sufficient to trap microscopic particles up to tens of micrometers in size and move them relative to their surroundings. For a particle to be trapped efficiently it must have a refractive index that is sufficiently higher than its surroundings. Trapped objects can be moved either by moving the microscope stage or by moving the trap position within the field of view. We have built a simple, compact, easy-to-use, safe and robust optical tweezer system that can be used with brightfield, phase contrast, differential interference contrast and fluorescence optics on a standard research grade inverted light microscope. Computer control of the trap position allows easy and accurate positioning of trapped objects. We have used this optical tweezer system in a number of fungal cell biology applications to trap and micromanipulate whole fungal cells, organelles within cells, and beads. We have demonstrated how optical tweezers can be used to: unambiguously determine whether hyphae are actively homing towards each other; establish microarrays of spores; move the Spitzenkörper within the hyphal tip and change the pattern of hyphal morphogenesis; investigate the effect of the wavelength of trapping light on growing hyphal tips; make force measurements; and deliver chemicals to localized regions of hyphae. We have also shown that spores that have been optically trapped exhibit no deleterious effects with regard to germination and germ tube growth.