

Dispensing Fungal Spores

Introduction

Isolation of individual germinating fungal spores is challenging due to the size and fragility of newly formed hyphae. Fungal spores can be quite large, with diameters greater than 30 μm and hyphae that can reach lengths of 100 μm or more. Because of these features, traditional cell sorters are not an ideal choice for sorting germinating fungal spores. In addition to having small dispensing nozzles that can be easily clogged by long hyphae, these high pressure systems (20–70 psi) can rupture the hyphae and greatly reduce the viability of isolated spores. Another pitfall is the risk of subsequent contamination of the device with non-germinating fungal spores, which are notoriously difficult to kill. Even with routine sterilization, there is a high risk of contamination if mammalian cell samples are sorted with the same machine.

Namocell's Single Cell Dispensers are gentle, low pressure systems (<2 psi) with large dispensing nozzles that can accommodate the long hyphae of germinating spores without clogging while also preserving cell viability after isolation. Additionally, Namocell's disposable microfluidic cell cartridges ensure no risk of sample carryover between experiments, removing the risk of contamination. Here, Namocell's dispenser was used to sort and isolate individual, fluorescently-labeled germinating fungal spores among non-germinating spores.

Methods

A mixture of germinating and nongerminating fungal spores (1:1) was prepared and germinating spores were labeled according to a standard protocol with wheat germ agglutinin (WGA)-FITC. Using the FITC detection channel as the trigger (533 nm), twelve individual FITC-positive spores were dispensed onto a glass slide placed on a 96-plate. Isolation of single spores in each droplet was verified via fluorescence microscopy.

Results

All twelve droplets possessed a single germinating fungal spore that was successfully isolated and dispensed from the initial mixed population (FIGURE 1). The dispensed fungal spores displayed intact morphology without any signs of rupture, indicative of healthy, viable cells. The longest fungal spores isolated reached over 100 μm in length and were successfully dispensed without clogging.

Overall, Namocell's single cell dispensers enable gentle isolation of germinating fungal spores. In addition, Namocell's disposable cell cartridges eliminate the risk of fungal contamination, such that mammalian cells could be sorted the next day using the same instrument.

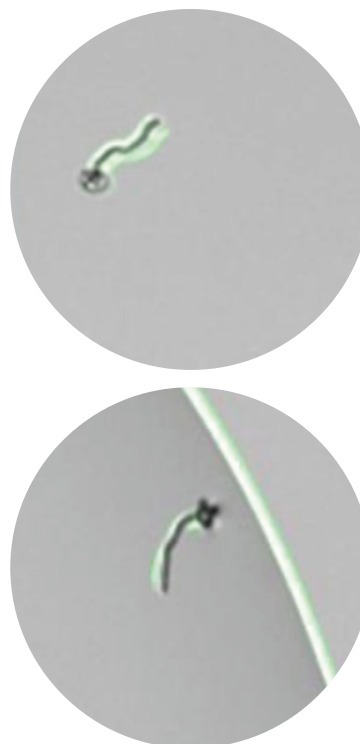


FIGURE 1. Single germinating fungal spores dispensed onto a glass microscope slide (images represent 2 out of 12 isolated spores).