Sample preparation for microscope imaging with filamentous fungi

Introduction

This protocol describes how to grow fungal mycelia in a way fit for 2D microscopy. The mycelium will grow (mainly) in a plane perpendicular to the vision axis of the microscope, enabling a full view of the entire mycelium.

Materials

- Fungal spore suspension
- MilliQ water
- Microscope glass slides
- Cover glass, if needed
- Liquid media with supplements (e.g. YEPD)
- Adhesive Microscope Slide Wells
  (We used “Grace Bio-Labs SecureSeal imaging spacer” for this)
- Paper towels, autoclaved
- Falcon tube 50 mL
- Calcofluor White (CFW) stain 1 g/L

Procedure

1. Prepare a dilution in MilliQ water from the spore suspension in order that a volume of 0.5 microliters contains 1 to 10 spores.
2. Adhere the adhesive in an autoclaved glass slide.
3. Add 7 microliters of liquid media onto each individual well.
4. Add 0.5 microliters of diluted spore suspension onto each well.
5. Lay the Falcon tube on the side. Place a rolled, wet piece of autoclaved paper towel into a falcon tube. It should lay vertically (from bottom towards cap).
6. Insert the slide on top of the wet paper. The well contents should be on the opposite side.
7. Incubate the microscope slides for desired time to see growth. For *Aspergillus niger*, we had reasonably sized mycelia at 24 hours for use with the 10x objective.
8. Add 0.3 microliters of CFW in order to visualise the samples in the fluorescent microscope. Set excitation to 380 nm and emission to 475 nm.