Fluorescence microscopy of Chlamydomonas reinhardtii - mCherry | Chlorophyll

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Abstract

This protocol describe the steps to perform *Chlamydomonas reinhardtii* fluorescence microscopy.

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Guidelines

Cells should be cultured to a late log phase to increase cell number for microscopy.

Before start

- Culture cells in liquid media

Protocol

Cell growth

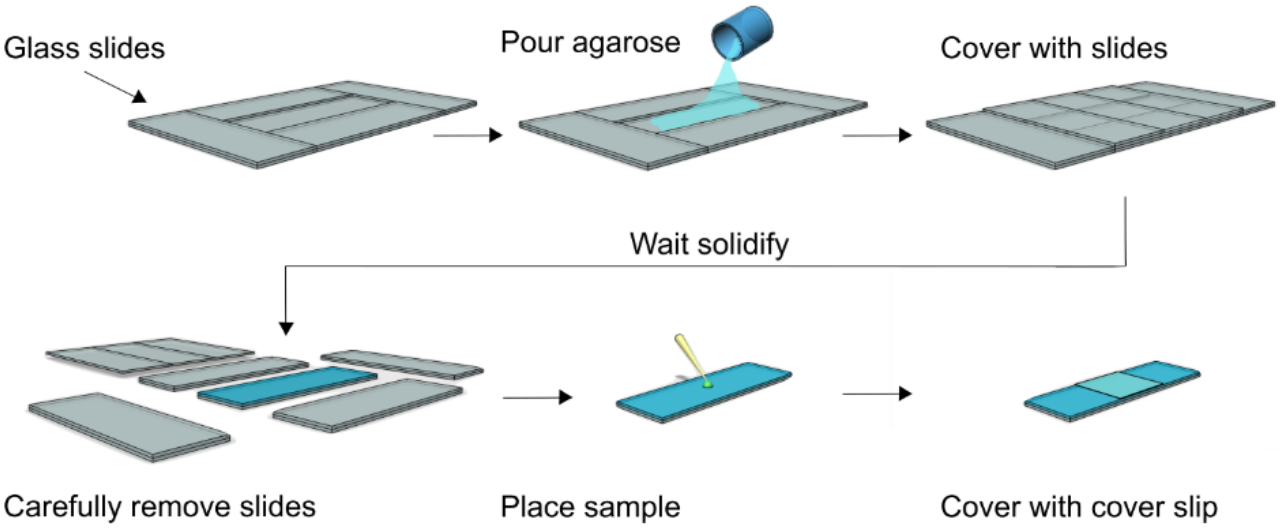
Step 1.

1. Grow cells to late-log phase density (1×10^7 cells/mL) in Tris-acetate-phosphate (TAP) medium at 25°C under constant illumination of 50 µmol photons/m^2s at 150 rpm on a rotary shaker

Agar pads

Step 2.

1. Prepare a 1% agarose TAP media, by adding sufficient agarose to TAP media and dissolve agarose by heating in a microwave.
2. Pour the melted media in a agar pad slide, or prepare a glass slide with a double adjacent slides barrier, forming a wall around the pad slide. Add slides on top of the poured media to allow formation of a smooth surface
3. Remove adjacent and top slides, and add 10 µL of culture to a desired spot on the solid media
4. Add a coverslip above the sample



Microscopy mCherry

Step 3.

1. Place slide on microscope
2. Use a argon laser at 543 nm to excite mCherry and a spectral detector set at 610-650 nm.
3. Record images.

Microscopy Chlorophyll

Step 4.

1. Place slide on microscope
2. Use a laser at 405 nm to excite chlorophyll and a spectral detector set at 680 nm.
3. Record images.

Stack image

Step 5.

For stack images use 0.4 µm steps per picture in z-axis