

Oct 11, 2022

# 🌐 Prepare agarose pad for microscopy (2022 iGEM)

Forked from [Fluorescence microscopy of Chlamydomonas reinhardtii - mCherry | Chlorophyll](#)

Team Fudan iGEM<sup>1</sup>

<sup>1</sup>Fudan University

1 Works for me

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[dx.doi.org/10.17504/protocols.io.8epv5jze5l1b/v1](https://dx.doi.org/10.17504/protocols.io.8epv5jze5l1b/v1)

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Team Fudan iGEM  
Fudan University

## ABSTRACT

This protocol describe the steps to perform fluorescence microscopy for *E. coli*

## DOI

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## EXTERNAL LINK

<https://doi.org/10.1371/journal.pone.0192433>

## PROTOCOL CITATION

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**protocols.io**  
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## FORK NOTE

## FORK FROM

Forked from [Fluorescence microscopy of Chlamydomonas reinhardtii - mCherry | Chlorophyll](#), Joao Vitor Molino

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71105

GUIDELINES

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

BEFORE STARTING

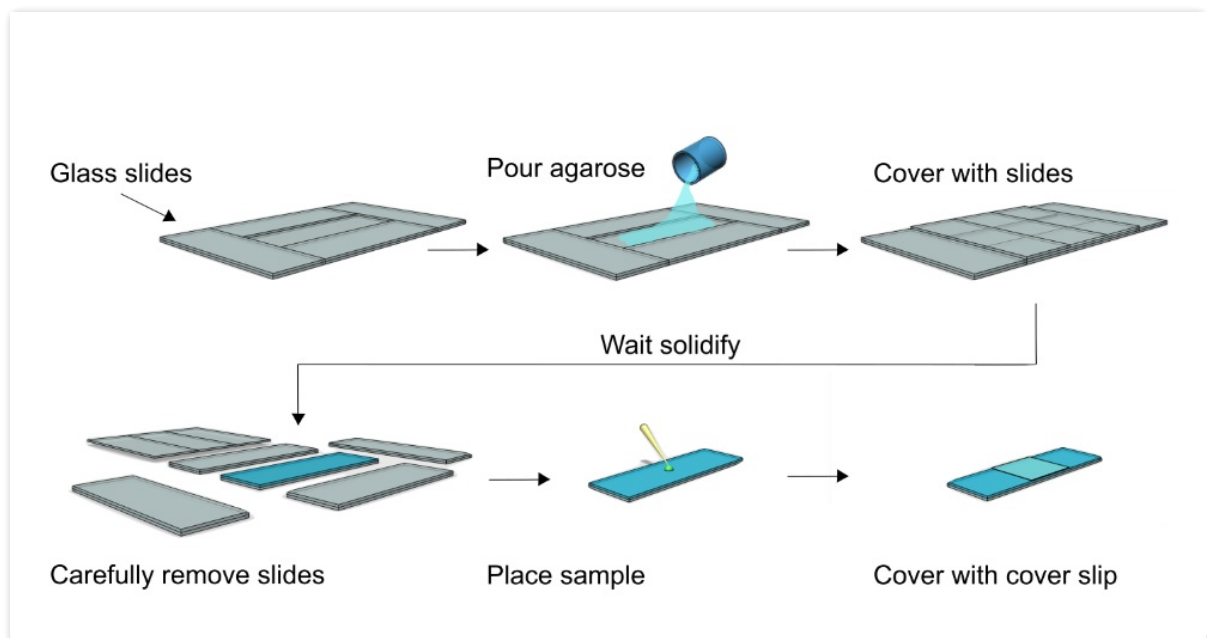
- Culture cells in liquid media

### Cell growth

- 1 Grow cells to late-log phase density (e.g.  $OD_{600} > 0.6$ ).

### Agarose pads

- 2 Prepare a 1% agarose in distilled water, by adding sufficient low-melting agarose to water and dissolve agarose by heating in a microwave for 30-60 seconds.<sup>16h</sup>



- 3 Prepare a glass slide with a double adjacent slides barrier (see below), forming a wall around the pad slide. Add slides on top of the poured agarose solution (1-1.2 mL) to allow formation of

a smooth surface. It takes time for 1% agarose solution to cool down to ~40 degree, and the solution is viscous but not runny.

4 

Wait for 45-60 minutes. Carefully remove adjacent and top slides, leaving the bottom slide.

5 Cut the pad into 15x15 mm square pierces, and carefully transfer the pad pierces to other cleared slide (left one agarose pad on the existing glass slide, if it was clean).

6 Add 2-5  $\mu$ L bacteria culture onto the agarose pad, and cover it was a 18x18 mm #1.5 square coverslip. Seal with wax if needed.

Microscopy GFP 5m

7 

- Place the slide onto the microscope stage, with the coverslip facing the objective.
- Use a laser at 488 nm to excite GFP.
- Record images.

5m

8 Analyze the images. For our microscope with 150x objective, the pixel size is 0.0806  $\mu$ m.