Media-Agarose Slides: A Mounting Method for Observation of Living Cells

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Often during light and fluorescent microscopy, one wishes to observe samples of cells (*e.g.*, yeast) grown in liquid culture. This is sometimes difficult as the cells can move around under the coverslip and a wet mount can dry out, killing the cells. Protocols in which the cells are resuspended in molten agarose results in cells that are in many different focal planes and often stressed by the high temperature of the molten agarose. This protocol describes a method of depositing small pads of growth media containing agarose onto slides, which eliminates these problems. Choice of growth media depends upon both the growth requirements of the cells and the requirements of the microscopy method. For example, yeast synthetic media works better for fluorescent microscopy than yeast rich media, because the yeast extract in rich media is autofluorescent in the green (fluorescein/GFP) channel.

1. Boil 25 ml of 1% agarose in the growth media of choice for one minute at full power in the microwave to dissolve. Swirl to mix.

2. Allow to cool for 1-2 minutes at room temperature. During this time, lay out several slides and open the box of coverslips.

3. Pipet the media-agarose onto the slide and **immediately** cover it with a coverslip. This will spread the agarose into a flat pad. The volume of media-agarose will depend on the size of the coverslip. For a standard 18 mm square coverslip, 50 µL is used. Continue making the pads until the agarose fails to spread to the edges of the coverslips, indicating it has cooled. Usually, at two pads per slide, at least 10 slides can be made.

4. Allow the pads to solidify. This takes about five minutes at room temperature.

5. Carefully pry the coverslip from the agarose pad(s) with a razor blade.

6. Add 3-5 µL of cell suspension or culture to the pad, re-cover with the coverslip, and observe. The use of the media-agarose pad allows observation of the sample at room temperature for at least four hours, allowing timecourse observations to be performed.

Notes on storage: unused slides can be kept for months as long as they are refrigerated and kept hydrated. I put them in a Rubbermaid container with a wet Kimwipe wrapped in Saran Wrap that has been slashed a few times with a razor blade. If the agarose pads adhere to the coverslip rather than the slide, they have dried out. The unused media-agarose solution can also be kept (covered) in the refrigerator. To re-use, add a small volume of media and re-melt in the microwave. This can be done 3-4 times. ■