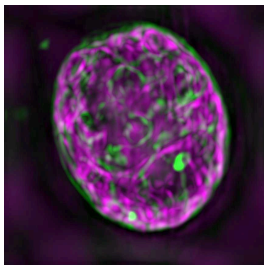
Nov 17, 2023

# Immobilizing cells for live imaging



DOI
dx.doi.org/10.17504/protocols.io.bp2l6xr15lqe/v1

Tara Essock-Burns[1], David Matus[1]

[1]Arcadia Science

Arcadia Science

Arcadia Science
Arcadia Science





**DOI: https://dx.doi.org/10.17504/protocols.io.bp2l6xr15lqe/v1**

**Protocol Citation:** Tara Essock-Burns, David Matus 2023. Immobilizing cells for live imaging. **protocols.io** **https://dx.doi.org/10.17504/protocols.io.bp2l6xr15lqe/v1**

**Manuscript citation:**
Avasthi P, Braverman B, Essock-Burns T, Garcia III G, MacQuarrie CD, Matus DQ, Mets DG, York R. (2023). Phenotypic differences between interfertile Chlamydomonas species. https://doi.org/10.57844/arcadia-35f0-3e16



**Protocol status:** Working
**We use this protocol and it's working**

**Created:** October 26, 2023

**Last Modified:** January 29, 2024

**Protocol Integer ID:** 89972

**Keywords:** live imaging, cells, agar, agarose, z-stack, immobilized, immobilization, Chlamydomonas, low-gelling agarose, immobilizing cell, confocal microscopy, algal cell, resolution confocal microscopy, cell, organelle, imaging, chloroplast, mitochondria, gelling agarose, agarose

## Abstract

This protocol covers the sample preparation for high-resolution confocal microscopy to visualize algal cells and their organelles (chloroplasts and mitochondria) by immobilizing them in agarose. The sample preparation includes preparing low-gelling agarose, staining the cells, and preparing the slides with live, but immobilized, algal cells.

## Image Attribution

Arcadia Science

## Materials

TAP medium (UTEX Media)
Low gelling agarose (Sigma-Aldrich A9414-50G)
1.5 mL microcentrifuge tube (clear) (VWR 20170-038)
1.5 mL microcentrifuge tube (black and opaque) (VWR 470228-440) or aluminum foil
Vital dyes of interest (PKmito Orange (Spriochrome))
18×18 mm #1.5 coverslip (VWR 48366-205)
25×75×1 mm pre-cleaned glass slide (VWR 48300-026)
parafilm (Amcor)
VALAP (1:1:1 mixture vasoline, lanolin, paraffin)
Forceps (EMS, 78317-2)
wax pencil (Phano china marker, 79, red)

## Troubleshooting

## Prepare the cells in appropriate medium

1 Scoop half of a sterile loop of cells (here we used *Chlamydomonas reinhardtii* and *C. smithii*) from a lawn plate and vigorously agitate the loop along the inside of a 1.5 mL microcentrifuge tube containing 500 µL of the medium for the experiment (TAP medium or dd$H_2O$).

2 Leave the tubes with cells in a tube rack on the bench overnight with a light source illuminating them under a 12:12 hr light:dark cycle.

## Prepare low-melt agarose in appropriate medium

3 To make 1.25% low-melt agarose in TAP medium (which we use for *Chlamydomonas* cells), measure 3.75 mg of low-melt agarose and dissolve into 3 mL of TAP medium in a glass culture tube, then place it on a heat block set to 100 °C until the agarose dissolves.

Note

You can maintain these small stocks of agarose at 45 °C and reuse them for one week. We recommend making fresh stocks after a week or if the agarose no longer appears transparent during imaging.

If you find that your cells do not stay immobilized during imaging, consider adjusting the concentration of agarose.

4 Once dissolved, move the tubes to a heat block set at 45 °C and let the mixture acclimate for at least 20 min.

## Staining the cells

5 Prepare a working stock solution of vital dyes in an appropriate medium. For PKmito ORANGE, we used 1:500 in TAP medium. For staining two species of *Chlamydomonas*, we prepared 1 mL of dye to split (500 µl for each tube).

6 Pellet the cells by microcentrifugation 2000 × g for 2 min. Remove the supernatant and discard it.

7 Add 500 µL of the working stock of dye to each tube and mix by gently pipetting up and down, resuspending the pellet.

8 Transfer the cell/dye mixture to an opaque microcentrifuge tube (or wrap the tube with foil) to protect it from light and secure the cap with Parafilm.

9 Place the tubes on a rotator to incubate with the dye for 45 min (at room temperature).

Note

Adjust the incubation time for different types of vital dyes, but always keep them protected from light.

10 After incubation, pellet and wash the cells twice with fresh TAP medium.

## Mounting the cells

11 Draw a small wax circle (using a wax pencil) on a #1.5 glass coverslip (18 × 18 mm) for each sample (two separate coverslips for two tubes).

12 After removing the supernatant following the second wash, resuspend the pellet in 25 µL of the warmed (45 °C) agarose mixture.

13 Pipette 1 µL of the agarose/cell mixture within the wax circle on the coverslip.

14 Using forceps, carefully flip the coverslip onto a glass slide and seal the edges with VALAP.

Note

If you find that the cells are not close enough to the coverslip for the working distance of your objective, consider gently and evenly pressing down on the coverslip with forceps before sealing with VALAP.