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Live cell microscopy sample preparation (yeast culture)

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Jakub Zahumensky[1], Jan Malinsky[1]

[1]Department of Functional Organization of Biomembranes, Institute of Experimental Medicine, Academy of Sciences of the Czech Republic, 142 20 Prague, Czech Republic

Jakub Zahumensky
Department of Functional Organization of Biomembranes, Insti...

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We use this protocol and it's working

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Abstract

In our approach, (cell-walled) cells in thick suspensions are placed on microscopy cover glass and overlaid by a small block of 1% agarose prepared either in buffer or cultivation medium identical to that of the respective cell culture, ensuring a close-to-natural environment. The cover glass is taped to a custom-made microscopy sample holder (Tinkercad file for 3D printing can be downloaded **here**). In this approach, the cells are immobilized on the cover glass just by mild mechanical forces, which does not induce a cellular stress response, as documented by normal actin structure in *C. elegans* (Chen and Pan, 2021) and by the absence of stress granules *S. cerevisiae* cells (Grousl et al., 2015; Vaškovičová et al., 2017). Furthermore, the cells spontaneously prefer a monolayer arrangement, i.e., they are mostly in the same focal plane. This makes microscopy imaging more efficient, saving both time and storage space. Since the agarose is permeable for small molecules, cells can be treated by directly adding chemicals onto the agarose block (in an inverted microscope) during time-lapse experiments (Grossmann et al., 2007; Grousl et al., 2015; Vaškovičová et al., 2017). Adding a cover glass on the other side of the sample holder (Fig. 2) prevents water evaporation and shrinking of the agarose block enabling long time-lapse experiments. The described arrangement thus represents an alternative to both surfaces coated with concanavalin A or poly-L-lysine, and expensive microfluidics systems.

Materials

Biological materials

The protocol was developed for S. cerevisiae and C. albicans cells and is described for these below. However, the analysis works with many cell types regardless of organism and/or tissue, and labeling (verified yeast suppliers are Euroscarf or Dharmacon; choose depending on your location).

Reagents

1. Reagent grade non-fluorescent agarose (e.g., Sigma-Aldrich, catalog number: A9539)
2. KH_2PO_4 (e.g., Lach-Ner, catalog number: 30016-CP0; Sigma-Aldrich, catalog number: P0662)
3. K2HPO4 (e.g., Lach-Ner, catalog number: 30060-AP0; Sigma-Aldrich, catalog number: P3786)
4. distilled/ionex H2O

Solutions

1. 50 mM potassium phosphate (KPi) buffer pH 6.3 (See Recipes)
2. 1% agarose in 50 mM KPi buffer pH 6.3 (See Recipes)
3. Optional: 1% conditioned agarose (See Recipes)

Recipes

	A	B	C
	Reagent	Final concentration	Quantity or Volume
	KH2PO4 (1 M solution in H2O)	38.75 mM	3.875 mL
	K2HPO4 (1 M solution in H2O)	11.25 mM	1.125 mL
	H2O	n/a	95 mL
	Total	n/a	100 mL

Table 1. 50 mM KP_i buffer pH 6.3. *store at 4 °C for up to three months*

	A	B	C
	Reagent	Final concentration	Quantity or Volume
	Agarose	1%	1 g
	50 mM KPi buffer pH 6.3	n/a	100 mL
			95 mL
	Total	n/a	100 mL

Table 2. 1% agarose in 50 mM KPi buffer pH 6.3 (for general microscopy experiments)

Heat the mixture in a microwave oven until all agarose dissolves. Let cool down to touch, then pour 9-10 mL into a 90 mm diameter Petri dish placed on a level surface. Wait until completely solidified (up to 30 min), then cover and seal the dish with Parafilm—store at 4 °C for up to a month. Before use, leave at room temperature for at least 20 minutes or put into an incubator at 30 °C for 5 minutes and cut with a scalpel blade into 1×1 cm squares. If you plan on adding chemicals directly to the agarose (e.g., during a time-lapse experiment), be precise with the cutting and measure the height of the agarose to calculate the volume of the agarose block.

Conditioned agarose (for specialized microscopy experiments, to be used immediately)
When harvesting (yeast) culture by centrifugation, remove 3 mL of the supernatant into a fresh 15 mL Falcon tube containing 0.03 g agarose. Close firmly, place in a tall glass beaker and heat carefully in a microwave oven on a low-intensity setting until all agarose dissolves (as the solution boils quickly, use multiple short intervals to prevent leaking of the solution out of the tube). Let cool down until you can hold the tube with bare hands, add chemicals (if desired) and pour into a 35 mm diameter Petri dish placed on a level surface (work quickly in this step, the agarose solidifies rapidly). Using conditioned agarose ensures that the environment in which the cells are grown is retained, minimizing stress exposure. This is especially important in situations when changes either in the composition of the media or its pH can result in significant changes in the localization, shape, or amount of the structure of interest, and also if the budding of yeast cells is to be monitored (Gournas et al., 2017; Grousl et al., 2015; Zahumenský et al., 2022)

Laboratory supplies

1. Petri dishes – 90 mm diameter (e.g., GAMA; catalog number: 400974)
2. Petri dishes – 35 mm diameter (optional) (e.g., Corning®, catalog number: CLS430165)
3. Erlenmeyer flasks (50-100 mL) for cultivation (e.g., PYREX®, catalog number: CLS49806L)
4. 15 and 50-mL Falcon tubes (e.g., Orange Scientific OrTubes, catalog numbers: 4440300 and 4440150)
5. 1.5 mL microcentrifuge tubes (e.g., Deltalab, catalog number: 200400P; Eppendorf Flex-Tube®, catalog number: 022364111)
6. 10 mL single-use pipettes (e.g., Orange Scientific, catalog number: PN10E1)
7. Compatible pipette tips (e.g., Gilson, catalog numbers: F161450, F161930, F161670; Eppendorf epT.I.P.S.®, catalog numbers: 022492004, 022492039, 022492055)
8. 20 or 50 mL single-use syringe (e.g., Chirana, catalog numbers: CH020L and CH050L)
9. Syringe filter with 0.2 µm pore size (e.g., Whatman, catalog number: 6896-2502)
10. Scalpel with no. 10 blade (e.g., Sigma, catalog number: S2896; Medin, catalog number: B397112910010)
11. Small spatula (home-made of old Western blot film works well, Fig. 1B)
12. High-transmission microscope cover glasses, 0.17 mm thick (e.g., Menzel Gläser 22×22 mm, catalog number: MZ-0025)
13. Lens cleaning papers (e.g., Kimtech Science, catalog number: 05511)
14. Scotch® Magic™ Tape
15. Permanent marker
16. ParafilmTM wrapping film

Equipment

1. Microscopy sample holders (Tinkercad file for 3D printing can be downloaded **here**)
2. Incubated orbital shaker (e.g., Lab Companion, model: SI-300R or N-biotek, model: NB205)
3. Centrifuge with a 1.5 mL microtube rotor (e.g., Labnet, model: SpectrafugeTM 24D)
4. Centrifuge with a 50 mL tube rotor (e.g., Hettich, model: Universal 320 R)
5. Micropipettes (e.g., Gilson, models: Pipetman P2, P200, P1000)
6. Pipette Controller (e.g., BrandTech, model: BRAND accu-jet®)
7. Laboratory scales (e.g., Kern, model series: 572)
8. Fluorescence microscope (e.g., Zeiss, model: 880 LSM; Olympus, model: FV1200 MPE or SpinSR10)
9. Microwave oven (e.g., ETA, model: Morelo)
10. Vortex (e.g., Labnet, model: VX-200 or Grant-bio, model: PV1)
11. Tube rack
12. A level surface
13. Personal computer/laptop

Graphical guide

1

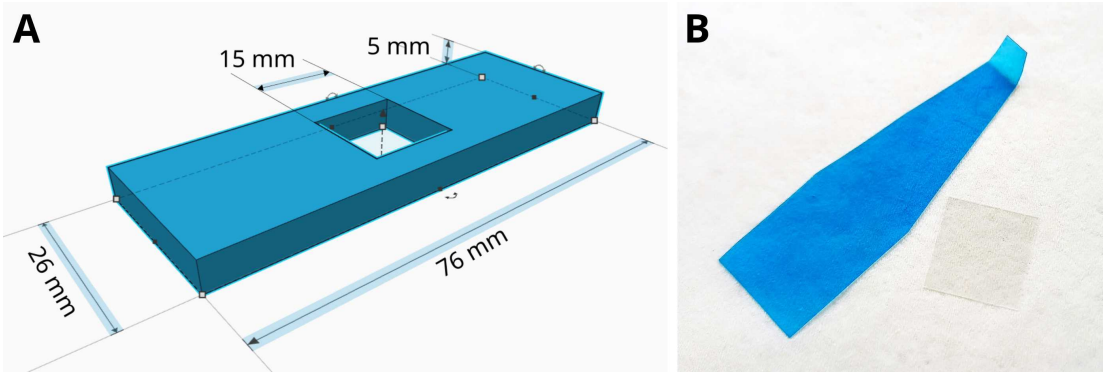

Figure 1. Equipment for microscopy sample preparation. A) Image of the custom-made microscopy sample holder; Tinkercad file for 3D printing can be downloaded here. B) A spatula made of old Western blot film (a 22×22 mm microscopy cover glass for scale).

2

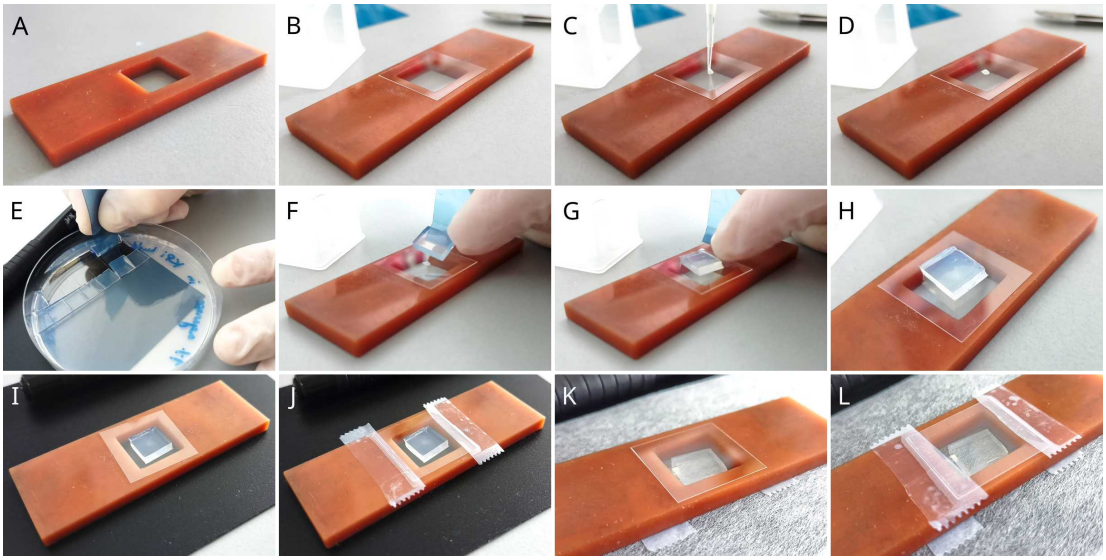

Figure 2. Microscopy specimen preparation. A) place cover glass on a level surface, e.g., the sample holder. B-C) pipette a small amount of cell suspension on the cover glass. D) use the spatula to lift a small block of precut agarose (roughly 1x1 cm) from the Petr dish. E-G) cover the cell suspensions with the agarose block. H+I) Turn the agarose upside down so that the agarose block is inside the square hole in the sample holder. J) tape the cover glass to the holder. K-L) for time lapse experiments, turn the sample holder upside down and tape another cover glass to seal the chamber.

Microscopy sample preparation

3 Cultivate cells under desired conditions.

4 Transfer the whole culture to a 50 ml Falcon tube (if not already used for cultivation) or remove 1 mL aliquot to a 1.5 mL microcentrifuge tube.

5 Centrifuge for 2 min at 1430 rcf at RT (room temperature).

6 Remove most of the supernatant by pipetting or decanting.

7 Vortex pellet in 3 one-second pulses (850 rpm).
Note: Alternatively, keep the supernatant and gently disturb the pellet by pipetting.
Note 2: If 1 ml of exponential culture is centrifuged in a 1.5 mL tube, repeat steps 2-5, pipetting the supernatant in step 4 both times.

8 Pipette 1 µL of the cell suspension onto a clean microscopy cover glass.

9 Cover the glass with a block of 1% agarose gel prepared with either buffer or cultivation media (see Recipes). Use a small flat spatula (Fig. 1B) to manipulate the agarose block.

10 Place the cover glass into the sample holder (Fig. 1A), with the agarose block inside the chamber (Fig. 2).

11 Tape the cover glass to the holder with Scotch tape and mark with a permanent marker if you have more than one sample.

12 *Optional:* If you are performing a time-lapse experiment and/or use a microscope stage incubator to control temperature, flip the sample holder upside down onto a lens cleaning paper and tape another cover glass from the other side (this prevents the agarose block from drying out during longer experiments)
Note: steps 6-10 are depicted in Fig. 2.

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