Culturing human HeLa cells under agarose for live microscopy studies

1- Grow human HeLa cells at 37 °C on 22 mm coverslip coated with poly-L-lysine in DMEM medium supplemented with 10% FBS the day before the experiment.

2- Prepare a 170 μm thick layer of agarose as following (see also top figure):

i) Place two rectangular coverslip fragments (obtained from a 25 mm^2 coverslip) on opposite ends of a slide, to act as spacers. Put a drop of PBS or water to stick the spacers with the slide.

ii) Using the heater, melt 0.1 g of low melting agarose (SIGMA cat # A-9414) in 5 ml of L-15 culture medium (serum-free).

iii) After heating, supplement the mixture with 10 % FBS and pipette the liquid agarose into the space between the coverslip fragments.

iv) Place another slide on top of the agarose to form a sandwich and wait until solidifies. This can be kept at 4 °C for a week in a humid chamber.

v) Carefully separate the two slides with a razor blade.

3- Cut a 0.5-1 cm^2 from the agarose layer and gently place it on top of HeLa cells grown the day before on the coverslip. Make sure to leave enough medium (follow step-by-step instructions from the figure).

4- Mount the coverslip in an open rose chamber and put small pieces of paper soaked with L-15 culture medium + 10 % FBS to keep the cells humidified.

5- Carefully add more L-15 culture medium + 10 % FBS to the cells.

6- Close the chamber with a cleaned 22 mm coverslip and observe the cells by DIC + fluorescence on the confocal microscope previously heated at 37 °C.