Instructions for confocal microscopy of cells in the Mimetix® scaffold (12-well format)

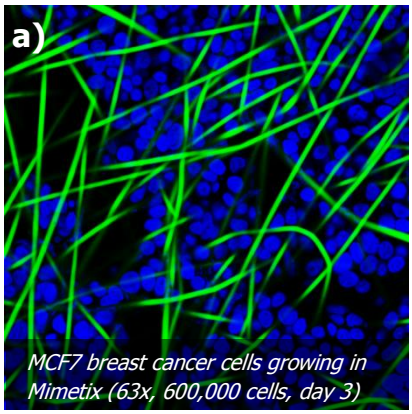
Confocal microscopy is a useful tool to obtain high-resolution images of cells within the Mimetix scaffold and investigate their depth profile. In the example protocol in the next paragraph, structures were stained with a) TO-PRO® 3 (nuclei) and b) Rhodamine 6G (fibres) or b) TO-PRO® 3 (nuclei) and Alexa Fluor® 488 Phalloidin (all from Life Technologies).

Cell seeding:

For a typical experiment using Mimetix in a 12-well plate format, prepare a cell suspension at a concentration of 100,000 - 500,000 cells/mL for short experiments (1-3 days), or 50,000 – 250,000 cells/mL for longer experiments (up to 7 days) and add 1 mL into each well. Allow cells to adhere for 24 hours or overnight.

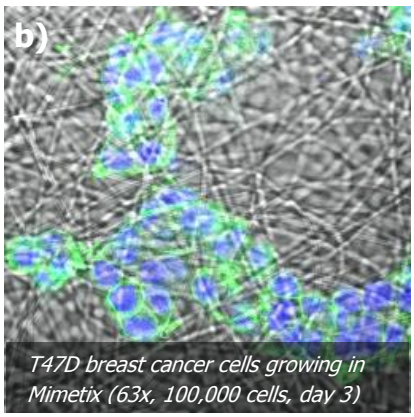
Cell staining:

1. To fix the cells in the scaffolds, aspirate medium and add 0.5 mL pre-warmed 4% paraformaldehyde in PBS to each well.
2. Incubate for 15-30 min, then aspirate paraformaldehyde, wash 2x with PBS and add 0.5 mL 0.1% Triton X-100 in PBS to each well to permeabilise cell membranes (only necessary when performing Alexa Fluor® Phalloidin staining).
3. Incubate for 5 min, then aspirate Triton X-100, wash 2x with PBS and add 0.5 mL 0.5% BSA in PBS to each well to act as a blocking agent (only necessary when performing Alexa Fluor® Phalloidin staining).



MCF7 breast cancer cells growing in Mimetix (63x, 600,000 cells, day 3)

4. Incubate for 30-60 min, then aspirate 0.5% BSA in PBS, wash 2x with PBS and add 200 µL Alexa Fluor® Phalloidin working solution into each sample.
5. Incubate for 30-60 min, then aspirate Alexa Fluor® 488 Phalloidin, wash 2x with PBS and add 1 mL TO-PRO® 3 working solution (1:10,000 in PBS) to each dish.
6. Incubate for 10 min, then aspirate TO-PRO® 3, wash 2x with PBS and 1x with water to prevent the formation of salt crystals.
7. Remove scaffold discs from wells and place on a glass slide, cell-seeded side facing up. Add a drop of mounting medium, place cover slip on top (dry off excess mounting medium with paper towel) and seal edges with nail varnish.



T47D breast cancer cells growing in Mimetix (63x, 100,000 cells, day 3)

8. Image stained cells within Mimetix by exciting Alexa Fluor® 488 Phalloidin at 488 nm using an Argon laser and detecting at 500-550 nm, Rhodamin 6G-labelled fibres at 543 nm using a He-Ne laser and detecting at 550-615 nm, and TO-PRO® 3 at 633 nm using a He-Ne laser and detecting at 650-710 nm.

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