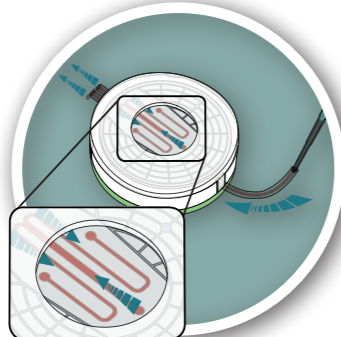




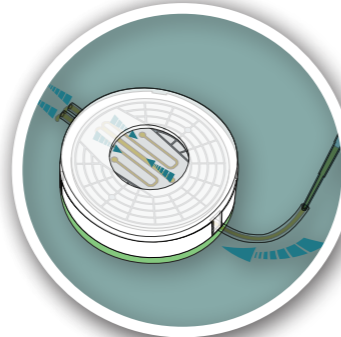
1. Equilibrate media

» Place cell media overnight in an incubator in a slightly open vial (for temperature equilibration and degassing).



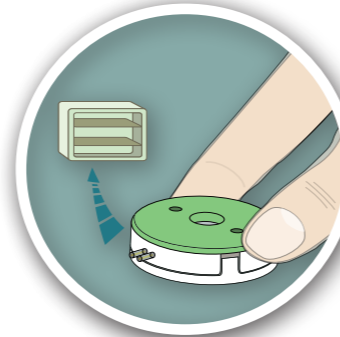
2. Apply coating

» CellDirector 2D is delivered uncoated (glass). Depending on the adherent cells, the assay needs to be coated prior to cell seeding.
» Slowly pipet 200 μ l of your coating solution into the outlet tube.
» Incubate at room temperature (10-60 min).



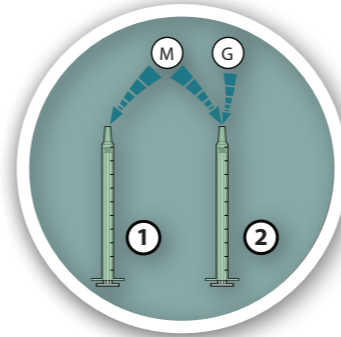
3. Cell seeding

» Prepare your cell suspension as usual (0.5-1x10⁶ cells/ml recommended for chemotaxis experiments).
» Slowly pipet 200 μ l cell suspension into the outlet tube.



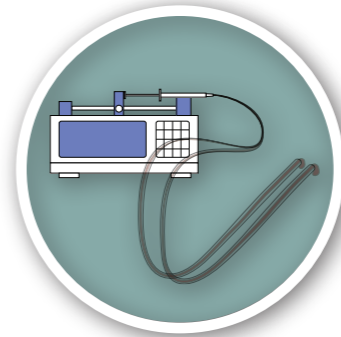
4. Adhesion of cells

» Place CellDirector 2D in a sterile petri dish and place in a humidified incubator.
» Incubate until the cells have adhered (60min, confirm with microscope).



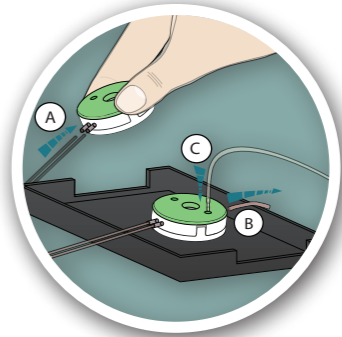
5. Syringes

» Fill syringe (1) with cell media (M), and syringe (2) with cell media and gradient substance (G).
» Connect the needles to the syringes and fill up the tubes.



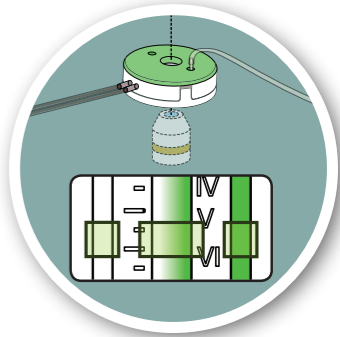
6. Syringe pump

» Fit the syringes into the syringe pump (e.g. Fusion 100, REF 90-001).
» Select the BD 1ml Plastic syringe in the syringe library (ID 4.78 mm).
» Start pump – ensure the tubes contain no air bubbles and media is exiting tube ends.
» Set flow rate to 1 μ l/min.



7. Start the experiment

» Tilt CellDirector 2D until inlet connectors are completely filled (A).
» Insert both tubes until fully bottomed and resistance is noticeable.
» Place CellDirector 2D under the microscope (CellDirector Holder, REF 70-001).
» Remove the outlet tube by pulling it gently (B).
» Connect the vacuum pump (C).



8. Image acquisition

» Set the positions from where to collect images (e.g. neg. control, gradient, pos. control).
» Collect images every 30 s (fast responding cells) up to every 10 min (slow responding cells).
» Continue to collect images during the entire experiment (30 min up to 16 h).