Written protocol for running dicty cells in the cell race chip

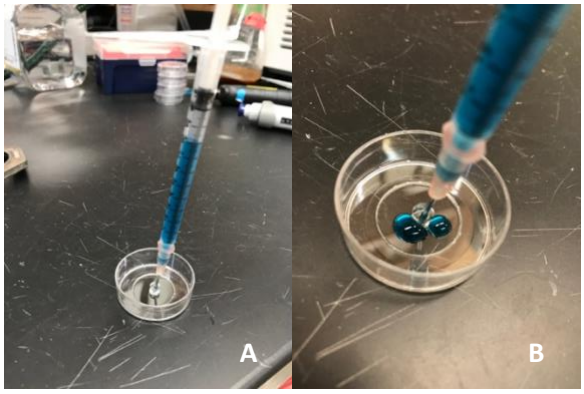
1. ***Starvation protocol***
2. ***Device priming with chemoattractant***
3. ***Cell loading***
4. ***Time-lapse microscopy***

1. Starvation protocol

1. Harvest cells from either a plate or shaking culture, use at least $4*10^6$ cells in total
2. Wash the cells twice in PB buffer
3. Resuspend the cells to a concentration of $2\text{-}3*10^7$cells/ml
4. Put the cells in an Eppendorf with a round bottom (2ml tube instead of standard 1.5ml)
5. Put the cells on a rotor or tumbler at about 20RPM for 6hrs
6. After 6 hrs spin down the cells and resuspend until you have a concentration of $1*10^8$cells/ml
7. Immediately load this solution into the prepared maze

2. Device priming with chemoattractant – using a Syringe

1. With a 1 mL syringe, first pull about 200 uL of air. Then, pull about 700 uL to 800 uL of chemoattractant. Add 18-gauge blunt end needle.
2. Holding the syringe upright so that the blunt end is facing up, press gently until liquid comes out the needle. This assures that all bubbles are gone.
3. Gently insert needle half-way into loading port of device, should be a snug fit and syringe should be able to stand on its own while in device **(Figure A)**.
4. Push syringe down loading about 50 uL-100 uL into device, with some force*. Solution should be coming out the side ports **(Figure B)**.
5. Looking under the microscope, you should see that some of the end chambers will be partially loaded, if the correct force was applied **(Figure C).** Too little force and chambers will remain empty. With some force, push down on the syringe in 50 uL "spurts". Leave a few seconds between pushes or the syringe will pop out due to much pressure. Keep the device under the microscope so you can observe the air pockets remaining get replaced by the solution.
6. Repeat until all chambers are full **(Figure E)**. Re-fill the syringe if necessary. BE CAREFUL to have the syringe loaded adequately enough so you are not pushing air into device**



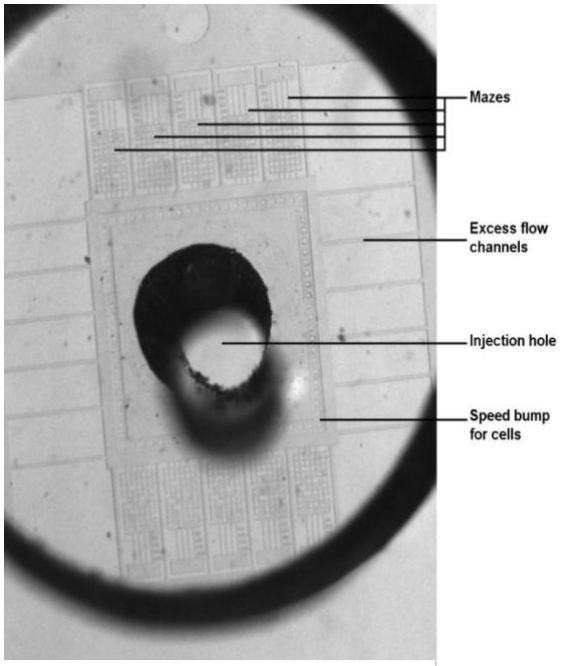


Figure 1-Overview of race chip

* Too much force may cause the device to pop off or completely be destroyed or cause the syringe to pop out splattering the solution everywhere.
** So you are not making massive amounts of chemoattractant you can use the extra solution that comes out of the device

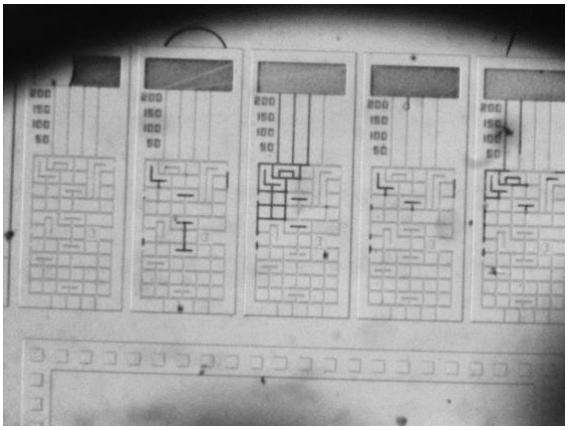


Figure 2- Incomplete priming, dark areas are air

3. Cell loading

1. Take 5 µl of the cell suspension and slowly inject into the maze using a thin tip on a 20µl pipette. You don't have to inject all! Stop when you see a white cloud of cells coming out from the sides. *Note: Do not be too forceful when pipetting in cells.*
2. Check the maze again if there are any air bubbles and if the cells were loaded

4. Timelapse imaging

1. Focus on 3 maze chambers (1x zoom in the software),
2. wait until the first cells have entered a maze to adjust the focus so you can best see the cells inside the maze, don't focus on the cells outside of the maze.
3. Start a movie, click on start experiment, log in to your cytosmart account, give your experiment a name and click on one frame every 10 seconds.
4. You can now start your experiment! It will continue making a movie for 4 hours. *Note: You can access these videos online on the cytosmart website and watch in real time as the race progresses.*

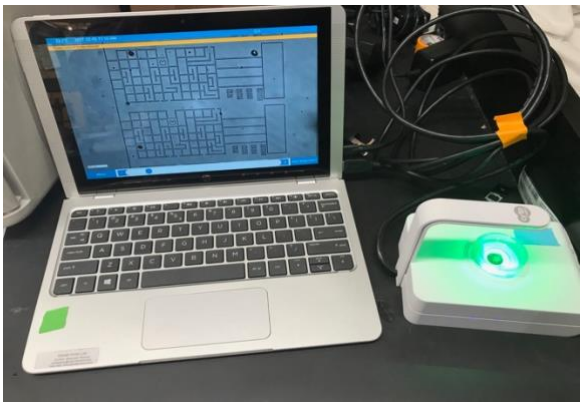


Figure 3-Setup of microscope with device

Some extra tips if you have trouble filling up the maze:

- *Some bubbles might take some time to go away, you might just have to wait*
- *Try to remove some chemo-atracctant from around the maze, creating suction on that side*
- *Apply more force on the syringe when loading*

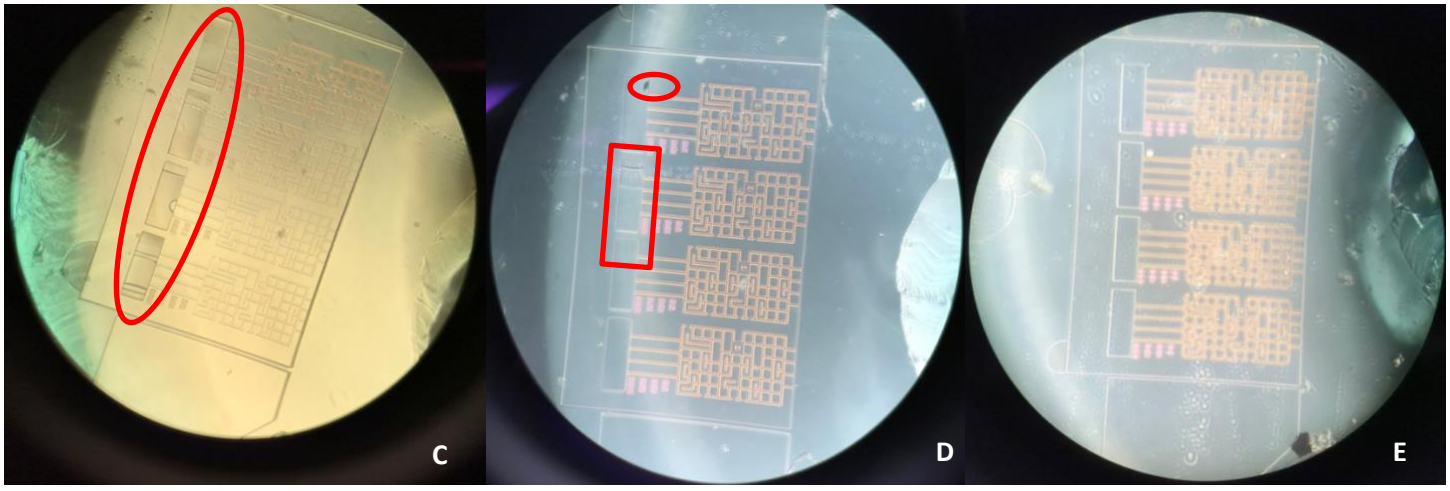


*Figure 4- **C**- incomplete priming of device. **D**- mostly complete priming of device. **E**- correctly primed device.*

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