Cell Selection Device

Work done by the hardware team of EPFL iGEM 2021 in collaboration with the KU Leuven team during summer 2021.

The aim of this device is to sort out cells accordingly to a defined parameter, such as the cells coming out of the device are optimized for a certain task or environment by directed evolution. Cell growth would be achieved in a bioreactor. Cell samples would be periodically extracted from the bioreactor, then analyzed and sorted by this device.

Introduction

Once a sample of cells has been extracted from the bioreactor, it enters the selection device. The selection device is governed by a microcontroller (MCU). The MCU receives an image as an input, treats the data contained in the image vector and finally sends the output signal selecting the best cells. It also switches between the elimination state and the recuperation state (Figure 1).

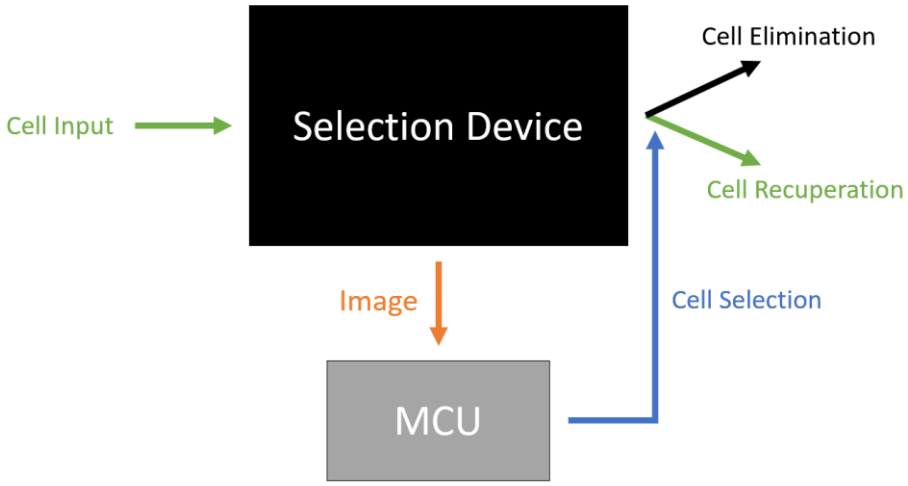

Figure 1: Blackbox Selection Device Schematic with MCU exchange.

The complete process flow of the selection device is as follows and is visually displayed on figure 2:

1. A sample of cells enters the selection device.
2. The cells are put into new media to minimize contamination during measurement.
3. The cells enter the wafer chamber.
4. Cells contained in droplets of media are individually isolated into micro-wells.
5. A waiting period for fluorescence starts.
6. An image is taken by the camera and is sent to the MCU.
7. The MCU selects the cells to eliminate.
8. The selected cells are eliminated.
9. The remaining cells are recuperated and are reinjected into a bioreactor.

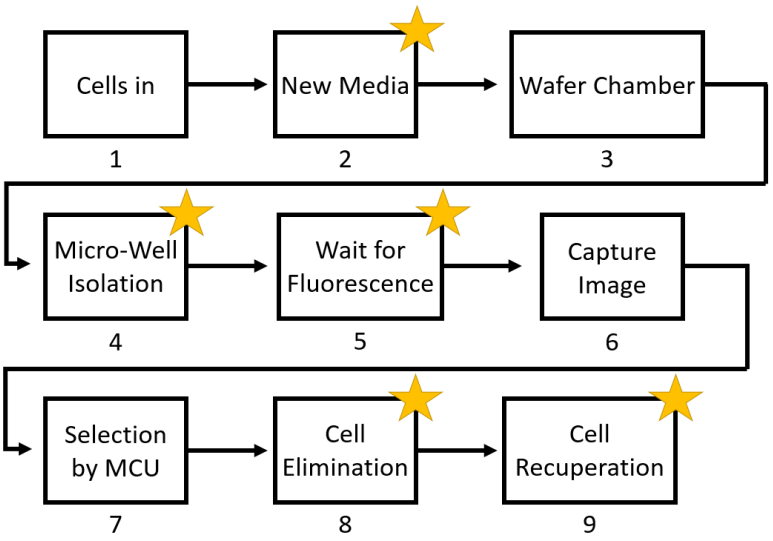

Figure 2: Cell Selection Flowchart

Detailed description of key steps in the process flow

Steps 2, 4, 5, 8 and 9 (starred blocks in Figure 2) required particular attention during the design phase:

Step 2: Introduction of cells into new media

The cells must be introduced into new media to avoid contaminating the measurement phase (step 5). This is because the media in which the cells grow in the bioreactor will be highly concentrated in the chemicals we wish to measure separately for each cell (see step 5).

To move the cells from the old media to the new media, we imagined a system with parallel tubes and an electric field (Figure 3). It is important that the flow is as laminar as possible to minimize mixing of new and old media. The process should also be fast enough to ensure minimal diffusion of contaminating chemicals into the new media.

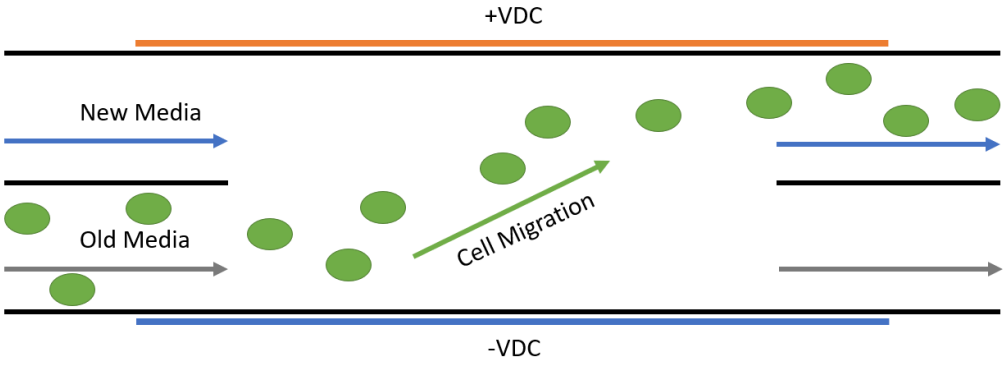

Figure 3: Schematic of cell migration into new media.

Step 4: Isolation of cells in micro-wells

Once the cells have entered the wafer chamber (named wafer chamber since it must be produced using cleanroom fabrication methods), they are separated using small electrodes (Figure 4).

Most of the wafer's surface being hydrophobic, hydrophilic compartments ensure that each cell is in a small droplet of water. Oil is then injected into the compartment to flush out all the remaining water, effectively isolating the cells and their respective water droplet (Figure 5).

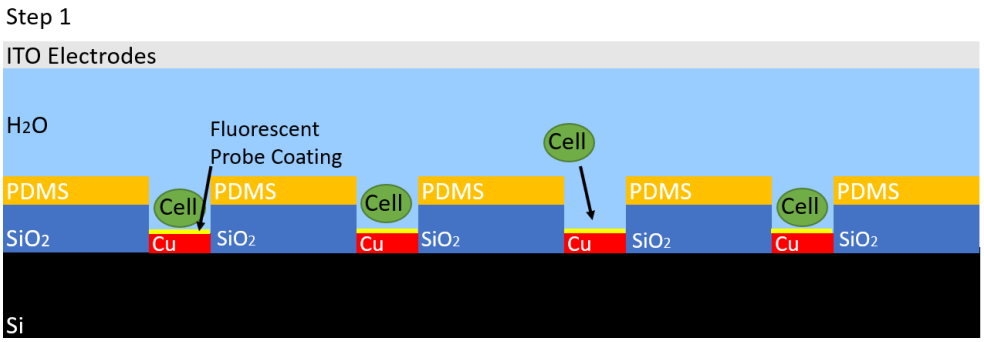

Figure 4: Cell Separation

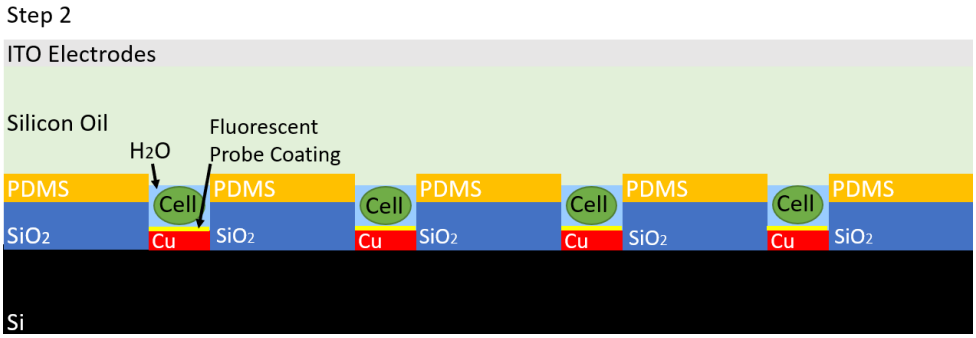

Figure 5: Cell and Droplet Isolation

Here are the main cleanroom fabrication[1] steps for this wafer:

1. Growth of the SiO2 layer by oxidation.
2. Application of positive photoresist by spin coating.
3. UV lithography with a prefabricated photomask.
4. Removal of the liquified positive photoresist.
5. Wet etching (BHF) of the SiO2.
6. Photoresist stripping.
7. Repeat steps 2-4.
8. Growth of copper electrodes and copper track by sputtering.
9. Photoresist stripping.
10. Repeat steps 2-4 but with a negative photoresist.
11. Growth of PDMS (1) (Polydimethylsiloxane) layer by thermal deposition (2).
12. Photoresist stripping.
13. Repeat steps 2-4.
14. Growth of Fluorescent Probe (3) thin film by chemical vapor deposition.
15. Photoresist stripping.
16. Inspection.

Step 5: Waiting period for fluorescence

Once the cells are isolated in their respective droplets, a waiting period begins so that the cells can produce and excrete the chemical compounds that will be detected and used to select the best cells. These reagents accumulate in the water droplet and react with the substrate, creating a fluorescent

[1] Inspired from the Microfabrication Technologies (MICRO-331) course taught by Prof. Jürgen Brugger and Prof. Martinus Gijs at EPFL.

effect. More production of the specific chemical means more fluorescence that can then be captured by the camera.

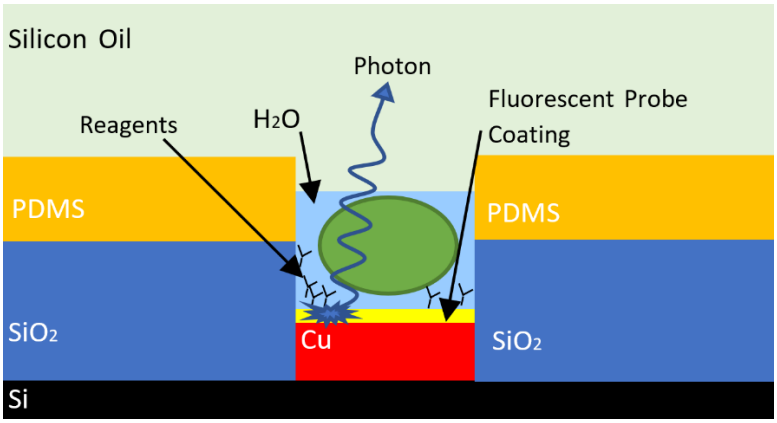

Figure 6: Schematics of chemical reactions.

Step 8: Cell elimination

The undesirable cells are eliminated by inverting the voltage of their respective electrodes and by flushing them out with water. The flow brings them towards the exit of the wafer, where they are sorted into the tube for elimination with an electric field.

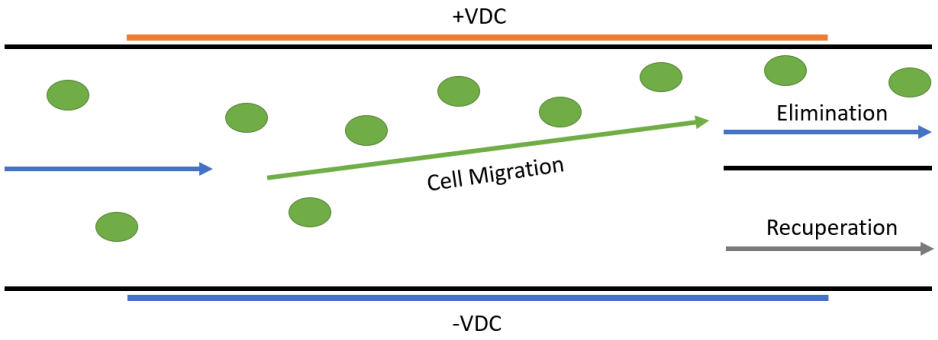

Figure 7: Cell sorting for elimination.

Step 9: Cell recuperation

The desirable cells are recuperated by inverting the voltage of their respective electrodes and by flushing them out with water. The flow brings them towards the exit of the wafer, where they are sorted into the tube for recuperation with an electric field.

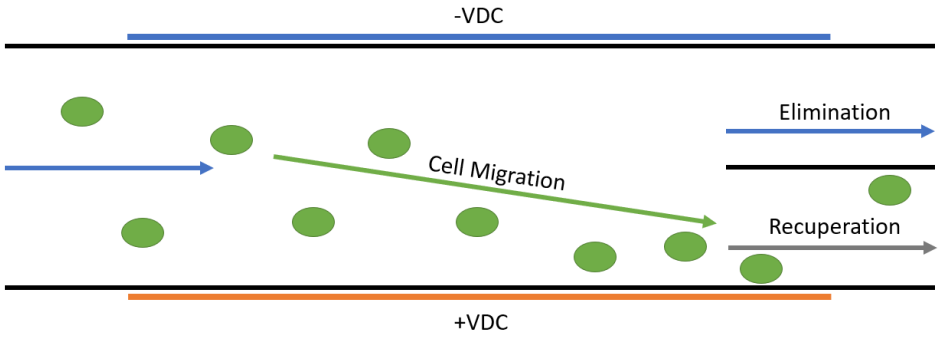

Figure 8: Cell sorting for recuperation.

References

(1) Materials for Microfabrication. *uFluidix*.https://www.ufluidix.com/microfluidic-technical-knowledgebase/materials-for-microfabrication/

(2) Han, S. W.; Kim, I. H.; Kim, J. H.; Seo, H. O.; Kim, Y. D. Polydimethylsiloxane Thin-Film Coating on Silica Nanoparticles and Its Influence on the Properties of SiO2–Polyethylene Composite Materials. *Polymer* **2018**, *138*, 24–32. https://doi.org/10.1016/j.polymer.2018.01.036.

(3) Fluorescent Probes - CH //www.thermofisher.com/uk/en/home/life-science/protein-biology/protein-biology-learning-center/protein-biology-resource-library/pierce-protein-methods/fluorescent-probes.html