

# Design and simulation of microfluidic device for metabolite screening and quantitative monitoring of drug uptake in cancer cells

Afia Asif <sup>1,†</sup>, Saed Khawaldeh <sup>2,3,4,†</sup>, Muhammad Salman Khan <sup>1,†</sup>, and Ahmet Tekin <sup>1</sup>

1. Graduate School of Engineering and Science, Ozyegin University, Istanbul, Turkey
2. Erasmus+ Joint Master Program in Medical Imaging and Applications, University of Girona, Girona, Spain
3. Erasmus+ Joint Master Program in Medical Imaging and Applications, University of Burgundy, France
4. Erasmus+ Joint Master Program in Medical Imaging and Applications, UNICLAM, Cassino FR, Italy

<sup>†</sup> Authors contributed equally to this work and they share the first authorship

E-mail: [afia.asif@ozu.edu.tr](mailto:afia.asif@ozu.edu.tr), [khawaldeh.saed@gmail.com](mailto:khawaldeh.saed@gmail.com), [salman.khan@ozu.edu.tr](mailto:salman.khan@ozu.edu.tr), [ahmet.tekin@ozyegin.edu.tr](mailto:ahmet.tekin@ozyegin.edu.tr)

## Abstract

Although liquid-liquid extraction methods are currently being applied in many areas such as analytical chemistry, biochemical engineering, biochemistry, and biological applications, accessibility and usability of microfluidics in practical daily life fields are still bounded. Suspended microfluidic devices have the potential to lessen the obstacles, but the absence of robust design rules have hampered their usage. The primary objective of this work is to design and fabricate a microfluidic device to quantitatively monitor the drug uptake of cancer cells. Liquid-liquid extraction is used to quantify the drug uptake. In this research work, designs and simulations of two different microfluidic devices for carrying out multiplex solution experiments are proposed to test their efficiency. These simplified miniaturized chips would serve as suspended microfluidic metabolites extraction platform as it allows extracting the metabolites produced from the cancer cells as a result of applying a specific drug type for a certain period of time. These devices would be fabricated by making polydimethylsiloxane (PDMS) molds from the negative master mold using soft lithography. Furthermore, it can leverage to provide versatile functionalities like high throughput screening, cancer cell invasions, protein purification, and small molecules extractions. As per previous studies, PDMS has been depicting better stability with various solvents and has proved to be a reliable and cost effective material to be used for fabrication, though the sensitivity of the chip would be analyzed by cross contamination and of solvents within the channels of device.

**Keywords:** Microfluidics; micro and nano fabrication; photolithography; drug uptake; cancer cells; multiphysics simulation; in vitro; metabolite screening

## Introduction

This article discusses the passive extraction operation where no chemical reaction occurs, furthermore, such kind of operations makes the delivery of material to be encouraged by the differences in chemical density between the two liquid stages. Particularly, when two unmixed liquids are in touch with each other, solute interchange up to the limit when the concentration proportions in the two liquids approach their stability gains. This method is surely reasonable for obtaining matter from a particular stage or decontaminate an element. Nevertheless, several motives are available to lessen the liquid-liquid extraction techniques.

Regular benefits of miniaturization are the depletion of instances number and the improvement in the movability possibility. Also, transport phenomena is quicker in micro-devices than in regular sizes devices, accordingly, it is predicted that liquid extraction requires a smaller amount of time to be acquired in miniaturized systems. This is based on the fact which assumes that diffusion occurrences (which normally restrain the movement in micro-devices) is

calculated as the square of the built device, and surely the lengths to cross are much smaller. This characteristic was presented lately in co-flow trigonometry, employing plug flow in capillaries or droplets, and also it has been applied in droplets which are confined in micro-chambers.

Several former studies in the past decades have described different implementation methods and usage of microfluidics devices. In 2013, David J. Beebe *et al.* [1] developed an enclosing abstraction for microscale capillary flow. This developed concept was called suspended microfluidics. Suspended microfluidics allows an advanced level of restraint over the channels working with several fluidic materials. In that system, the fluidic materials flow happened in an unclosed micro-tunnel, where the fluidic materials were "suspended" between double or more air atmospheres or between an air and another unmixable liquid. In 2014, David J. Beebe *et al.* [2] presented new techniques, which made the investigation of the fetal Leydig group of cells in tissue culture easier. Furthermore, those tools made the utilization of the micro-atmospheres to be possible. Their objective in this work was to exploit micro-technology to find out fetal testis steroidogenesis which were molecular level intents and as a result this lead to stop and cure illnesses of masculinization which affects both fetal and adult male health. They contributed further in 2015 [3] by developing a microfluidics method to study the effects of soluble factor signaling on endothelial tubule formation, an important step in establishing an *in vitro* indicator of angiogenesis.

Delai Chen *et al.* [4], described the design of the "Chemistrod", which is the droplet microfluidics system for exploiting and obtaining molecular level waves with good spatial and temporal resolution. A great contribution was made by Rustem F. Ismagilov *et al.* [5], by presenting the novel idea of SlipChip in 2009. SlipChip is a system allowing to implement multiplexed practical tests and it also guarantees a control over the material available inside, given that it is a device based on the plugging concept. It opened the new avenues of research in microfluidics by initiating the ideas of multiplex solution extractions. Another unique service has been provided by Rustem F. Ismagilov *et al.* [6], by working on the Digital PCR on SlipChip. Digital PCR method was not only used for measuring the numbers of nucleic acid molecules, but also it was used to remark uncommon cells, measure the numbers of DNA and RNA and quantifying the performance of SlipChip with a great reliability on the single-molecule reactivity of PCR.

Methods, which rely on the droplet microfluidics concept also show a model for detailed checking, offering expanded throughputs, decrease specimen sizes and one-cell examination capabilities. Eric Brouzes *et al.* [7] presented a droplet microfluidic development, which enabled large-throughput checking of mammals' cells. Their design used a 2-level approach where every assay was

compartmentalized in watery micro-droplet (1 pL to 10 nL) encircled with an unmixable oil. The benefits of this micro-droplet approach including separating the droplets from the environment, avoiding the possibility of pollution, and finally providing a quick and coherent fusion for the compounds. Margaret Macris Kiss *et al.* [8] described a sustained-flow actual-time PCR device, which produces discontinued, pL scale PCR reactions in a maintained flow of motionless fluorinated oily liquid. To address the importance for large-throughput and actual-time PCR, a PDMS microfluidics device was designed, this chip was disposable and could be used to allow a sustained-flow, droplet-type PCR interaction and amplification.

In 2008, Pascaline Mary *et al.* [9], elaborated the research work on the liquid-liquid extraction, which became one of the motivation to devote services in this field. They firstly offered a fully descriptive study - in microfluidics context - about the relocations available between moving micro-droplets and another outer level. Ultrahigh-throughput screening in the microfluidics, which are based on droplet concept for directed evolution was carried out by David A. Weitz *et al.* [10], where they reported the use of pL size watery droplet distributed in a fixed unmoving oil as reaction channels; where all of them had only one yeast cell which displayed the variations on the enzyme's exterior. They further sorted the droplets at speeds of several hundred by second, enabling checking a library of several samples in nearly 10 hours, however, they only used reagent volume of < 150 L in total.

In this research work, two simulation designs of two different microfluidic devices are proposed for carrying out multiplex solution extractions in an easy format. The first proposed design has a long channel (1000  $\mu\text{m}$  length) connected with a small one (300  $\mu\text{m}$  length) which form a device with two inlets and one outlet. Fluids flowing in from the two inlets are diffused all over the junction and getting mixed in the long channel, which shows a substantial potential for screening sample contents. While in the second device, two straight channels serve as separate compartments for both drug and media, respectively with an additional cavity at the bottom for keeping the cells. These simplified miniaturized chips would serve as suspended microfluidics metabolites extraction platforms, and would be fabricated by making PDMS molds using soft lithography.

## Materials and methods

The designs of the devices presented in this research work is inexpensive and simplified. The basic working principle of both the devices is hydrodynamic diffusion based extraction of the complex sample into the desired fluid. The proposed microfluidic devices exhibit structures which are easier to fabricate than the other traditional devices presented in earlier studies. Moreover, having a capability of mixing fluid together in the channel shows a substantial potential for

screening sample contents. Diffusion and extraction of fluid is carried out by altering the materials depending upon their concentration and viscosity. Here, vegetable oil and water are the fluids used to test the proposed design using COMSOL Multiphysics®. The ultimate objective is to use these models for culturing cancer cells and extracting metabolites in the sample using chemo-attractants or specialized drugs to test further for quantifying their drug intake.

Briefly, first, the cancer cells are cultured in the long channel for the first device design and in the bottom micro-cavity for the second design by injecting them through one of the inlets, while a nutritional medium is flowing in from the second inlet. Outlets would be closed to provide some time for cells to bind themselves to the surface of the long channel. After some time, the outlets would be opened to drain all the fluids in the channel. The next step is to inject the specific amount and type of drugs into the channel for cancer cells exposure while closing the single outlet and the two inlets. The cells would take some amount of time to absorb certain amount of drugs. Once that period is over, the rest of the liquid is taken out as a first testing sample for liquid chromatography – mass spectrometry (LC-MS) analysis. As a last step, the fresh medium is injected from the inlet to collect the popped-out metabolites from cells as a result of drug exposure in order to confirm the quantitative results once again using LC-MS analysis.

The materials used for the microfluidics chips are SU-8 photoresist compound, PGMEA developer, PDMS base and agent. The fluids for testing simulations are vegetable oil and water, while the real time application materials are targeted as cultured cancer cells, specialized drugs, and a growth medium.

### Implementation and Experimentation

Several geometries with different dimensions were initially designed and optimized in order to come up with suitable and easy to fabricate structures. The proposed devices structures are modelled in COMSOL using the following major design steps and characteristics.

#### Defined Geometry

As stated above, the geometries are initially designed in COMSOL in the MEMS based microfluidics module. Two designs for this purpose are created and are shown in Figure 1 and Figure 2.

For Figure 1, the depth, width and height of the long channel created are 100 μm, 1000 μm, 100 μm, respectively, while the width, length and height for the shorter channel are 300 μm, 100 μm, 100 μm, respectively. Similarly, for second design in Figure 2, the top and middle channel has the depth, width and height of 500 μm, 100 μm, 100 μm, respectively and the bottom cavity has dimensions of 100 μm width, 100 μm depth and 25 μm height.

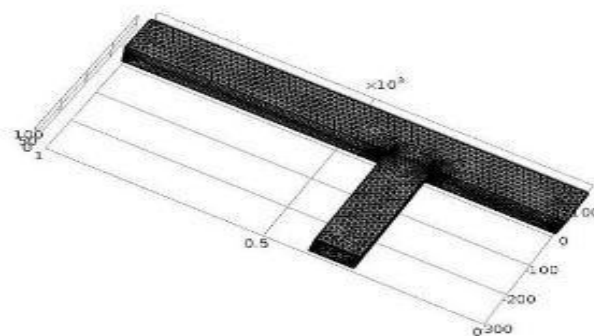


Fig. 1: The mesh geometry of the device 1

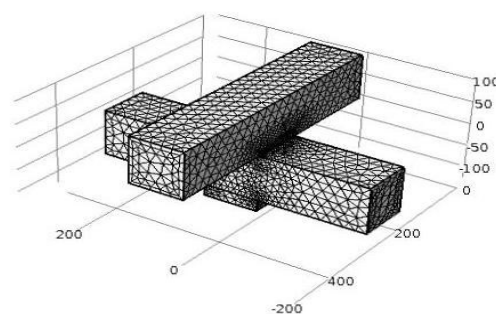


Fig. 2: The mesh geometry of the device 2

#### Defined Physics

In this section, the physical parameters such as concentration, velocity and diffusion constants of the fluids used in the channels, are given. Physical parameters are tuned for the different used materials and then optimized to get the desired results.

Whenever mass transportation of a dissolved species (solute species) is considered in a gas mixture, concentration gradients usually cause diffusion. If there is bulk fluid motion, convection also contributes to the flux of chemical species. Therefore, the equations below find the combined effect of both convection and diffusion. For diluted species:

$$\mathbf{N}_i = -D_i \nabla c_i + c_i \mathbf{u}$$

$$\frac{\partial c_i}{\partial t} + \nabla \cdot \mathbf{N}_i = R_i$$

Alternation in concentration in any moment, because of convection, is calculated using equation below:

$$\left( \frac{\partial c_i}{\partial t} \right)_{\text{conv}} = -\nabla \cdot \mathbf{N}_{i,\text{conv}} = -c_i \nabla \cdot \mathbf{u} - \mathbf{u} \cdot \nabla c_i$$

where  $c_i$  represents fluid concentration (mol/m<sup>3</sup>) and  $\mathbf{u}$  represents compound speed (m/s).

The first part of the equation equals to zero when an incompressible fluidic compound flows as a response for

conservation of fluidic compound mass. Availability of a certain solution to the convection formula provided an exact initial concentration profile  $c_0(r)$ , and a steady speed  $u$ :  
 $c(\mathbf{r}, t) = c_0(\mathbf{r} - \mathbf{u}t)$

**Mask design**

After deciding the final geometry and drawing its model in COMSOL, a photo mask is designed for the fabrication of the device by photolithography. Figure 3 below shows the CAD mask of device 1.

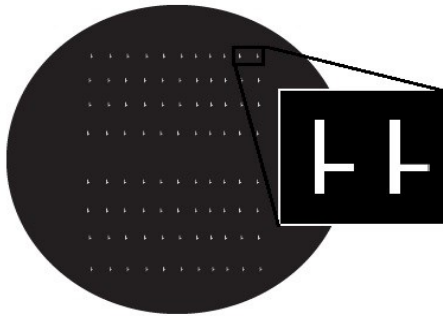


Fig. 3: CAD mask for device 1

**Microfabrication**

Fabrication of these devices using photolithography and soft-lithography techniques could be carried out with simple steps as explained further. Briefly, for this purpose, a very good resolution laser printer was used for the generation of an acetate mask. The mask was designed using the Adobe Illustrator CAD tool; this approach was adopted to minimize the mask-producing cost as much as possible.

The mask would be used in hard contact with the substrate for photolithography while using SU-8 photoresist compound to develop a negative "master", which has patterned photoresist on the silicon wafer. Positive replicas with embossed channels were then fabricated via casting PDMS onto the "master". Figure 4 presents the flow diagram of several fabrication milestones, while the overall experimental steps are mentioned in Figure 5.

Using PDMS provided various benefits such as exceptional flexibility, exceptional transparency, low cost, and easy and quick fabrication procedure. PDMS is hydrophobic; therefore, it supports water-in-oil admixtures. Plane slabs of PDMS are used by bonding them together to close the channels in order to enable fluid flow through them. Figure 6 shows the dimensional details of device design 1.

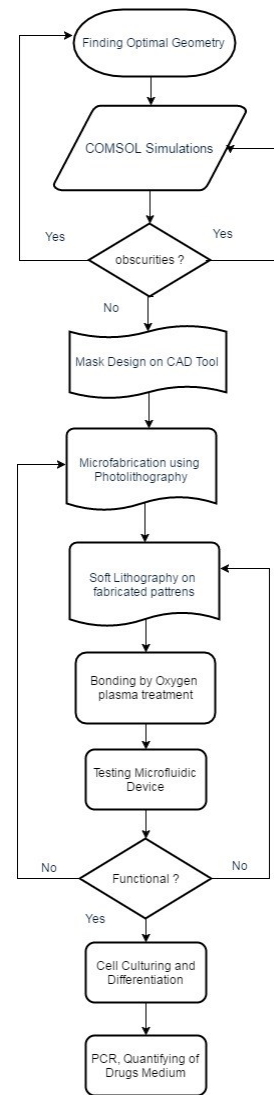


Fig. 4: Flow diagram of fabrication milestones

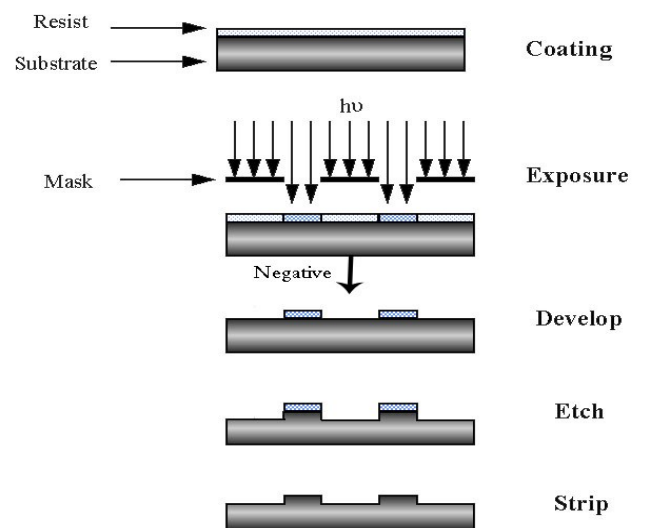


Fig. 5: Process steps of fabrication

practically realizable and also provides large surface area for culturing more cells and higher cells-drug interaction.

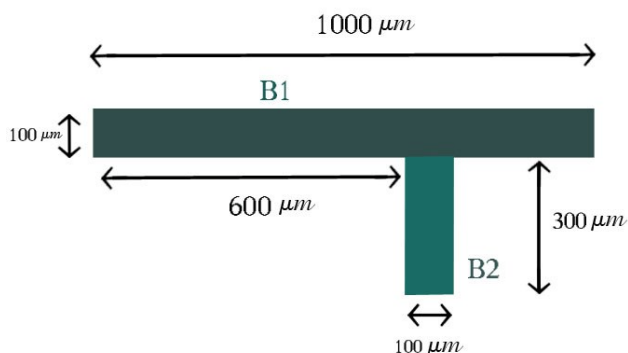


Fig. 6: Top view of Designed geometry for device 1

### Results and Discussion

As the concentration of the fluid changes (i.e. drugs in practical domain and vegetable oil in simulation testing domain), the impact on the sample fluid (i.e. cancer cells and water in simulation testing model) changes. Also, change in diffusion of the sample into another fluid increases with the increase of concentration level of the fluid. Below are the simulation results, which have catered interaction and diffusion between the fluids. Figures 7, 8 and 9 show the concentration profile, velocity profile and contour pressure profile of the fluids throughout the channels for the first design.

In this section, the second design and its simulation results for the multilayered device is discussed. The dimensions were already discussed in earlier sections. Below are the results of the concentration profile, contour pressure and fluid velocity as shown in figures 10, 11 and 12.

Figure 10 shows the drug introduction in the first channel, which results in the change of concentration in the middle channel due to the drug flow. From this, we can conclude that the drug is distributed to the bottom cavity and hence can interact with the cultured cancer cells.

Figure 11 describes the pressure gradient over the channels, it can easily be seen that gradient is higher at the inlets and that it decreases as the fluid covers distance in the channels and diffuses to the bottom cavity.

In Figure 12, the velocity at the upper channel is higher where the drug is pumped in from the inlet. From the velocity profile, it is seen that velocity is lower at the side walls due to collision of fluid with boundaries. As the drug travels into lower channels, the velocity is reduced.

As in comparison for two simulation designs, it was observed that although the second device provides explicit storage as a small cavity for cultured cells under the channels but it still lacks in providing the flexibility for simplified practical design because of the multi-layered structure. This design with channels on different levels would not only increase the cost for the fabrication steps but would also raise the cost for two different photo-masks. To overcome this limitation, the first simulation design is preferred to proceed for practical fabrication, which is

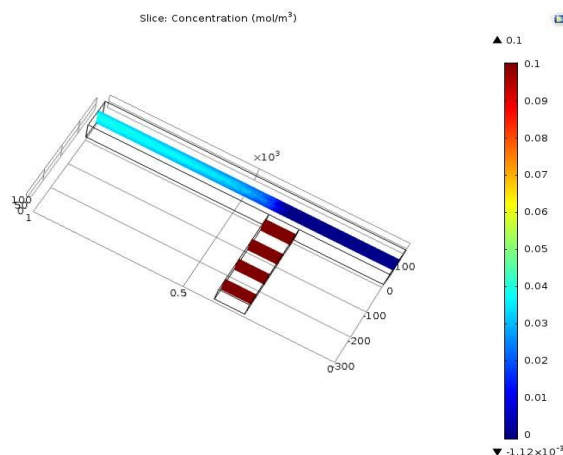


Fig. 7: Design of Concentration profile of the fluids for device 1

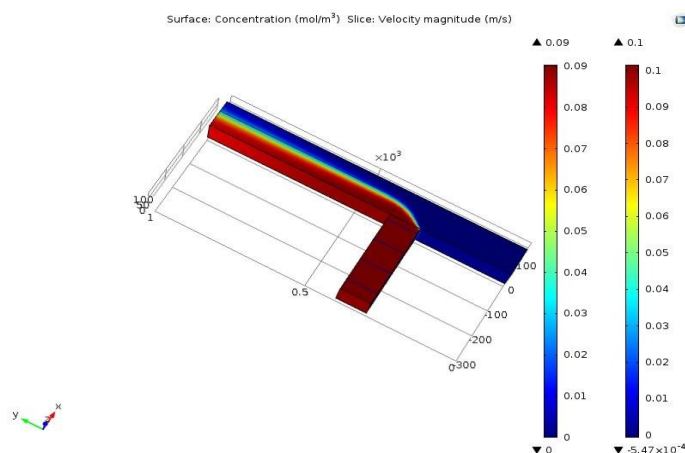


Fig. 8: Design of Concentration and Velocity Profiles of the fluids for device 1

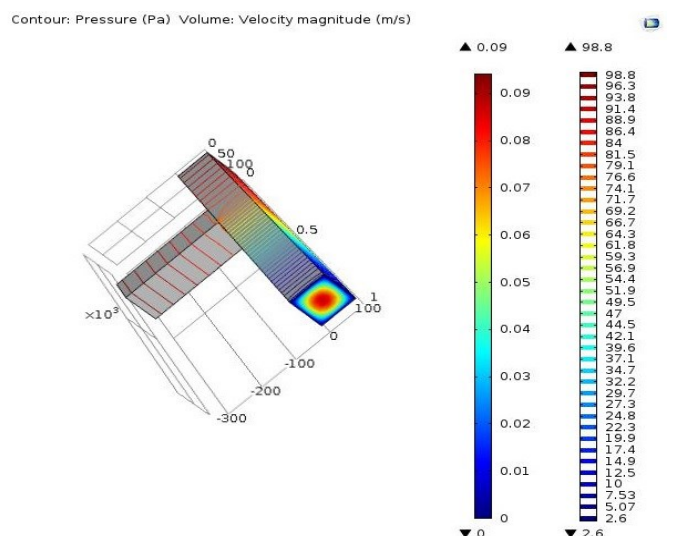


Fig. 9: Design of Contour Pressure profile of the fluids of device 1

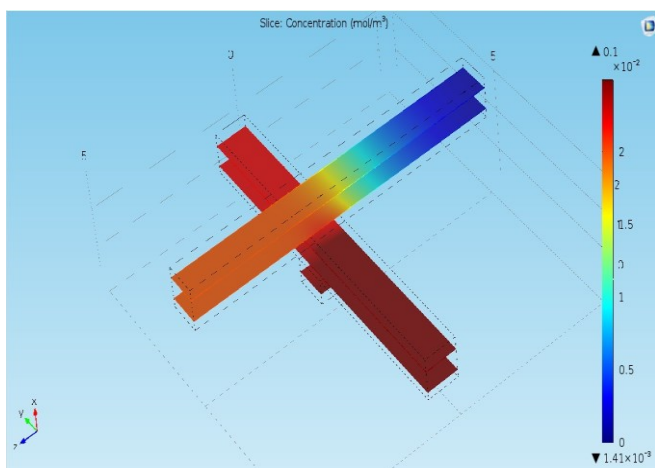


Fig. 10: Design of Concentration profile of the fluids for device 2

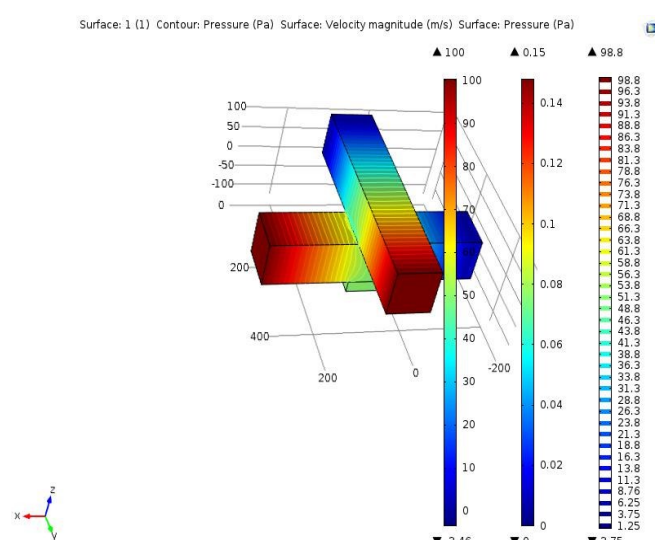


Fig. 11: Design of Contour pressure profile of the fluids for device 2

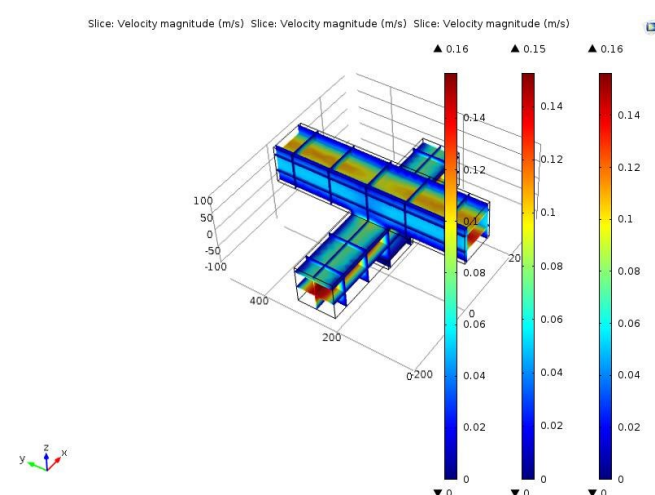


Fig. 12: Design of Velocity profile of the fluids for device 2

cells; testing different kinds of drugs and obtaining very accurate quantitative results. This device provides a great contribution towards studying the responses of cancer cells to cancer drugs. Moreover, it allows scientists to perform their experiments in a clean environment; it provides them with more precise results; it gives them the opportunity to determine the most and least efficient cancer drugs, which will contribute significantly to cancer studies; and it will be very useful in pharmaceutical industry.

In the future, the device structure can be improved by introducing more channels to provide more rooms or compartments for multiple interactions on the same chip.

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### Conclusion and Future Work

The design of our device 1, can easily be modified to perform more than one experiment at the same time. Furthermore, it has the following potentials: testing different kinds of cancer

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