MICROFLUIDIC DEVICE FOR BLOOD ANALYSIS

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ABSTRACT: The scientific work presents aspects regarding the functioning and modeling of a lab-on-a-chip microfluidic device used for blood tests where the leukocyte count is followed, which informs us about the state of the immune system. Aspects regarding the stage of development of lab-on-a-chip devices are presented, extremely useful in the current conditions of the pandemic due to the rapidity of providing analysis results and the opportunity to perform certain determinations, impossible with current conventional equipment. AutoDesk INVENTOR Professional software was used to model the device and COMSOL Multiphysics software was used to simulate the finite element operation of a circuit variant, as well as its geometric optimization. Research has been carried out on the components of the device, such as the pressure system and the counting system. The conditions for the execution of the device on a millimeter silicon wafer were created using photochemical microtechnologies.

KEY WORDS: leukocytes, microfluids, photochemical microtechnologies.

1. Introduction

The abbreviation for Micro Electro Mechanical System is "MEMS" (Micro Electro Mechanical System), which was officially adopted by Dr. Albert P. Pisano in 1989. He used the term "MEMS" to describe the resonant structure made as a frequency stabilizer. [1]. In the early 1990s, a micro-analytical system, also known as a "chip laboratory", was proposed. Due to the micro-scale, the fluid flow in the microfluidic device has different characteristics, ranging from 0.1 μm to 1 mm [2].

In this study, we analyzed the realization of the flow circuit on a silicon plate, to obtain a MEMS type microfluidic device used to determine the number of leukocytes in a blood sample, which are an indicator in determining the state of the immune system.

2. The product strategic marketing

Table 1. Restrictions for fabrication the product prototyping

Restrictions for fabrication the product prototyping	
R 1: To be a product which has the smaller dimensions than the classics products	*R 5:* To have the lowest cost.
R 2: To have a simple constructive form;	R6 : To be able to be reused
R3: Be able to quickly interpret the results collected	R7 : To have easy maintenance
R 4: To have a large market sales;	R8 : To choose the optimal circuit

Data collected from potential customers

The issue of the analysis time of the microfluidic device was analyzed again and it was necessary to update and resend the previous questionnaire to the respondents. The following questions have been added to the existing questionnaire:

What would be the maximum waiting time for interpreting the results?

What do you think is the most important parameter that influences the reaction time of the device?

Also, in order to have a correctly interpreted answer and to help completed the device as well as possible, a new market segment was introduced in the selection matrix and more exactly the companies that deal with the manufacture of medical equipment.

Data collected from potential customers

The questionnaire used for the market research in the case of marketing the "Microfluidic Device" was made exclusively online. The questions added above have been interpreted in the figures below.

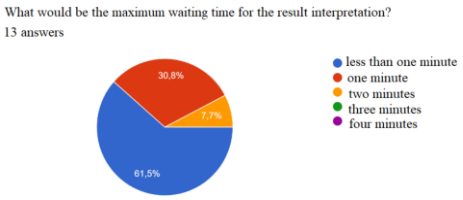


Fig.1 Graph waiting time

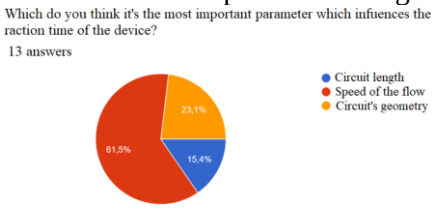


Fig. 2 Graph response time

The questionnaire guide together with the two interpreted requirements are presented in Table 2.

Table 2. Interview guide

No. crt	Question	Customers statement	Need interpreted
1	What would be the maximum waiting time for interpreting the results?	○ In less than a minute ○ One minute ○ Two minutes ○ Three minutes ○ Four minutes	○ The need for as little analysis time as possible
2	What do you think is the most important parameter that influences the reaction time of the device?	○ Circuit length ○ Flow rate ○ Establishing relative importance Circuit geometry	○ The need to choose the most concrete parameter that best influences the analysis time of the device.

Hierarchy and relative importance

Following the study of the answers and the interpretation of the clients' needs, a grouping of the main needs was made, and then their relative importance will be established. Table 3 shows the relative importance, giving the grades 1 to 5 depending on the importance considered.

Table 3. Establishing relative importance

Client requests	Relative importance
The microfluidic device for the analysis of leukocytes in the blood is portable and easy to store	5
The microfluidic device for the analysis of leukocytes in the blood is reusable	5
The microfluidic device for the analysis of leukocytes in the blood is easy to use	4
Microfluidic device for blood leukocyte analysis has a prompt response in interpreting the results	5
Microfluidic device for blood leukocyte analysis provides precision in the analysis of collected samples	5
The microfluidic device for the analysis of blood leukocytes can be used in the medical industry	4
The microfluidic device for the analysis of blood leukocytes can be used in any environment being independent of the workplace	5
The microfluidic device for the analysis of leukocytes in the blood has affordable price for the current medical market	3

3. Product approval

- the overall dimensions of the microchip - it is checked if they allow the assembly with the display device and the pressure pumps. Depending on the geometry of the microchip, the sample has certain flow velocities during the analysis, pressures on the path traversed by the sample and a certain pressure in the capture chamber;
- the structure, size and hardness coefficient of the support plate, which helps to stabilize and protect the geometry of the microfluidic chip;
- use of the outer plate, which helps to protect the silicon microchip and its stability and fixation on the support plate;
- use of a counting sensor for the purpose of counting leukocytes in the blood sample inserted into the silicon microchip circuit.
- checking the volume of blood needed to analyze and number the blood sample;
- testing the density and kinematic viscosity characteristics of the lysis liquid used;

4. Internal organization for marketing

The delivery sector - one of the most important departments, given that the activity carried out by it, ensures the connection between the manufacturer and customers, is the interface with the beneficiaries, it's efficiency depending on customer satisfaction and consequently turnover. In view to determine the efficiency of the product delivery activity, the cost involved in the delivery to a sample of 10 medical laboratories located at various distances from the point of sale was analyzed.

The analyzed parameters were the delivery destinations, the distances to the delivery place, the delivery times, the quantity to be delivered versus the capacity of the transport machine (weight and volume to be transported), the delivery hours taking into account the traffic on the selected routes.

Hypotheses:

No. customers =10; No. ordered devices =95; Transport capacity information:

Table 4. Transport capacity information

Device weight	7 kg
Transport machine capacity (kg)	1000 kg
No. pcs. maximum that can be transported depending on the maximum weight	142 pcs.
Device volume 277 x 276 x 270 mm	0.02064 m3
Transport machine capacity (volume)	2 m3
Nr. pcs. maximums that can be transported depending on the volume	pcs.

Two delivery options were analyzed:

1. Delivery according to the ranking of the orders versus optimized delivery of all orders received / day
2. Delivery with own transport versus rented transport.

   4.1. Analysis presentation: Delivery according to the ranking of the orders - the transport of the order is carried out punctually, with the main purpose of obtaining customer satisfaction through the short delivery time, on the principle of "first come, first served"

Table.5 Customer distances

No. crt	Customer	Route (round - trip)	Quantity ordered	Distance (km)	Time (min)
1	Sante Clinic	University Politehnica of Bucharest- > Sante Clinic	10	2.6	7
2	Synevo	University Politehnica of Bucharest- > Synevo	20	2.8	11
3	Sfânta Maria Clinic	University Politehnica of Bucharest- > Sfânta Maria Clinic	10	2.6	18
4	Regina Maria Hospital	University Politehnica of Bucharest- > Regina Maria Hospital	5	3	11
5	Medlife Clinic	University Politehnica of Bucharest- > Medlife Clinic	10	4.2	18
6	OneLife Clinic	University Politehnica of Bucharest- > OneLife Clinic	5	3.2	9

No. crt	Customer	Route (round - trip)	Quantity ordered	Distance (km)	Time (min)
7	Cris Medical Clinic	University Politehnica of Bucharest- > Cris Medical Clinic	5	3.6	13
8	Sanador Hospital	University Politehnica of Bucharest- > Sanador Hospital	10	3.4	16
9	Apaca Polyclinic	University Politehnica of Bucharest- > Apaca Polyclinic	5	1	7
10	Emergency hospital of Bucharest	University Politehnica of Bucharest - > Emergency hospital of Bucharest	15	3.6	10
		Total	95	30	120

The transport routes are presented below:

-Optimized delivery of orders- the aim is to streamline costs while meeting customer expectations, respectively the delivery time communicated to them (24 hours from the date of the order).

Table 6. Optimized order delivery

No. crt	Customer	Route (round - trip)	Quantity ordered	Distance (km)	Time (min)
1	Synevo	University Politehnica of Bucharest- > Synevo	20	2.2	6
2	Sfânta Maria Clinic	Synevo - > Sfânta Maria Clinic	10	0.35	2
3	Regina Maria Hospital	Sfânta Maria Clinic - > Regina Maria Hospital	5	0.45	2
4	Medlife Clinic	Regina Maria Hospital - > Medlife Clinic	10	0.9	3
5	Sante Clinic	Medlife Clinic - > Sante Clinic	10	0.85	3
6	Cris Medical Clinic	Sante Clinic - > Cris Medical Clinic	5	7.1	16
7	Onelife Clinic	Cris Medical Clinic - > OneLife Clinic	5	1.2	4
8	Sanador Hospital	OneLife Clinic - > Sanador Hospital	10	1.1	4
9	Emergency hospital of Bucharest	Sanador Hospital - > Emergency hospital of Bucharest	15	1.1	3
10	Apaca Polyclinic	University Politehnica of Bucharest- > Apaca Polyclinic	5	1.8	7
		Total	95	17.05	50

1. Delivery with own transport versus rented transport.

In calculating the costs, the driver's salary, fuel and other expenses related to the transport car (depreciation, tax, insurance, repairs) were taken into account. The expenses of the car represent a percentage of 3% in total salary and fuel expenses.

Table 7. Own transport costs

Costs of company transport	
Wage	3000 lei wage + employer taxes/160 hours = 18.75 lei/hour * 2 hour = 37.5 lei
Fuel	7 lei/liter * 15 km* 10 liters consumption/100 km = 10.5 lei
Car expenses	(3000 wage + 10.5 lei/day *20days)* 3/100=96.3 lei/month/20 days = 4.815 lei/day
Total internal transport costs / day	52.815 lei/day

In the calculation of the expenses with a rented car, a cost of 1.5 lei / km and 2 lei / stop was taken into account.

Extern transport costs/day = 1.5lei/km*15+2 lei/stop*10 customers=42.5 lei/day (1)

5. Computer modeling and simulation of microfluidic flow in the micro-electro-mechanical device

Computer simulation has become an essential part of science and engineering, it is the technique that verifies the correctness of selected materials in the construction of new parts / devices, as well as production / manufacturing technologies. Thus, the dedicated Comsol Multiphysic finite element modeling software was used and then successively the working modules were selected: Fluid Flow, Single Phase Flow and Laminar Flow for modeling and simulating the flow of substances used in the microfluidic device. The input data are presented in Table 8.

Table 8. Input data for microfluidic modeling and simulation

Flow proportions	
Blood	50 µm/min
Lysis solution	1600 µl/min
Lysis stop solution	265 µl/min

The simulation of the flow rate of the fluid mixture is presented in Fig. 3

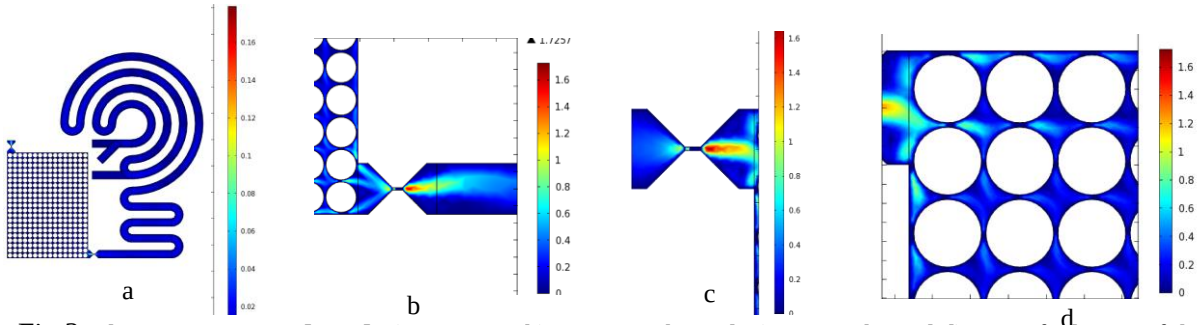


Fig 3. Flow rate variation [m / s], a) overview, b) counting channel, c) output channel d) magnified view of the selection and capture chamber

The velocity of the fluids collected from Comsol at points in the median flow circuit, due to the parabolic distribution of the laminar flow, was very low on the straight lines and much higher on the curved lines or the reduced flow section (Fig.3). The objectives of the blood sample analysis with lysis and lysis stopping substances have been achieved. Calculating the length of each circuit in relation to the corresponding values of the average speed, the travel time for circuit 1 was 2,831 s less than the characteristic value of 3 s required for the proper red cell lysis process. For circuit 2, the speed was 46,289 s. This is higher than the reference value for stopping lysis, which is 30 s.

In order to reach the final version above, we started from an initial version that went through various changes shown in the following images:

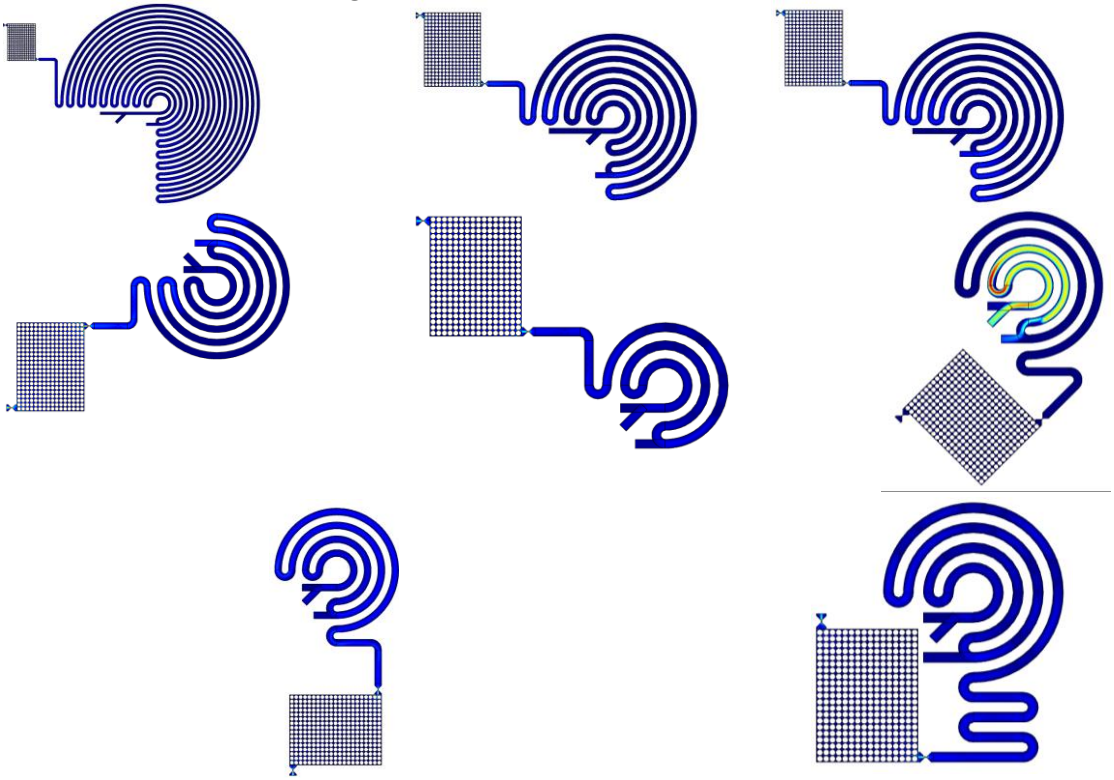


Fig. 4 Modeling models of the circular microfluidic device

6. Manufacture of microfluidic chip

Figure 5 shows the execution drawing of the microfluidic chip.

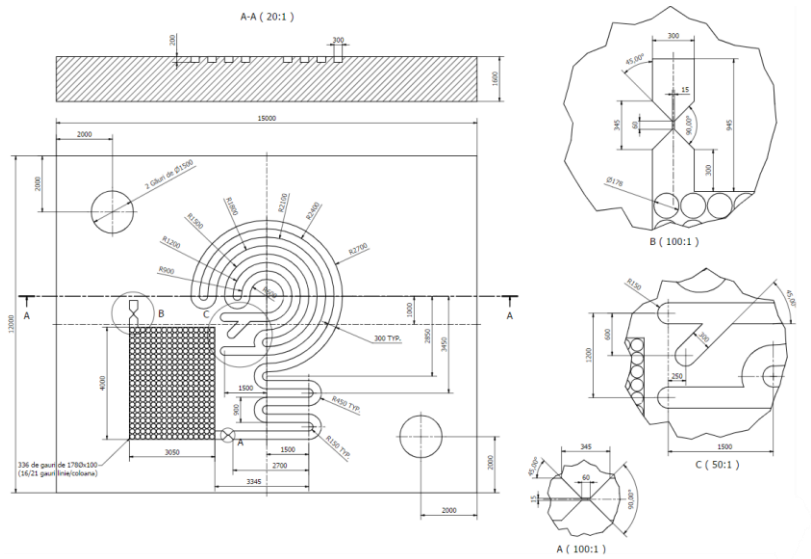
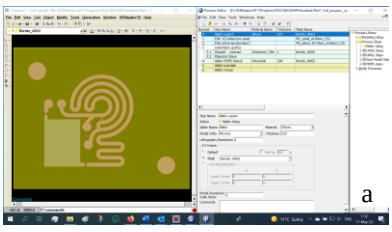


Fig. 5 Microfluidic circuit drowing

The figures below show the steps taken to make the microchip. SEMulator3D software was used to verify the correctness

a. Data input for 3D modeling: on the left DXF format graphic file, on the right standard processing steps for technology flow modeling.
b. Choice of chip holder 15x12mm2, thickness G525µm
c. 3D modeling result for 100µm depth corrosion, channel and other 100 µm depth tank
d. 3D modeling deepening pillars at a depth of 100µm and another 100µm in channel depth
e. Highlighting (for graphic purposes) the contrast substance of the geometries dug by corrosion in their 525 µm thick monosilicon support
f. Casting using the mechanical mask of the PDMS following the geometries excavated in the subducted monosilicon
g. Adhesive PDMS adhesion to subducted monosilicon
h. Duplicate 31 chips of 15x12mm2 - 4inch monosilicon wafer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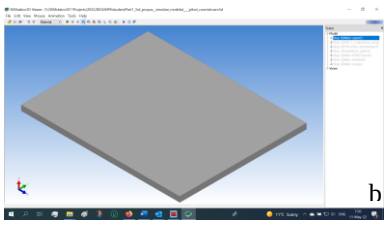
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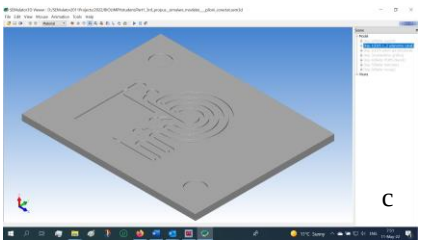
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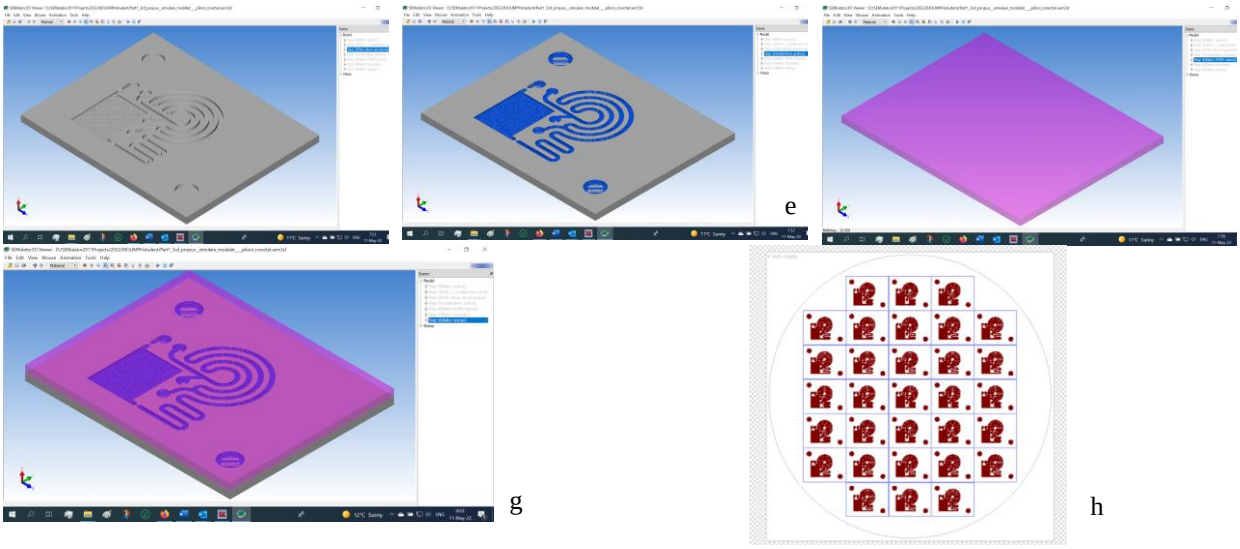

Fig. 6 Stages of microfluidic chip manufacturing in SEMulator3D software

7. Cleaning the device

Several cleaning steps are normally required during the production stage of the device, as well as before its actual use, to ensure that the chips and connectors are properly cleaned. After precise connections and experimental preparation, the last thing a microfluidics user wants to observe on its microscope is a clogged channel with debris that could have been avoided. If this happens, the options to remove contamination inside microchannels and connections are unfortunately limited. [5]

A classic and easy method of cleaning the microfluidic device is the manual one which includes the following series of steps:

- Manual application of a detergent solution for 5 minutes (removal of small particles)
- Manual application of a methyl alcohol (methanol) for 5 minutes
- Manual application of acetone for 5 minutes (for the removal of organic residues)
- Manual application of demineralized water for 5 minutes
- Drying the device with an oven or laboratory hob (Figure 7)

Fig. 7. Laboratory electric hob

Another method is to put these liquids in glass or plastic cups and place in an ultrasonic bath for a few minutes. These actions include manipulative steps that make the process tedious and time consuming. [5]

Using bags can reduce the time and the complexity of cleaning microfluidic chips and other laboratory tools (connectors, tweezers, glass slides prior to plasma bonding to PDMS). Additionally, we have reduced the amount of liquids used routinely.[5]

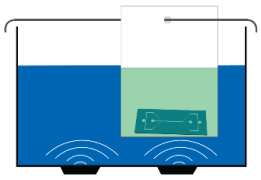

Fig. 8 Sketch of the use of a bag for indirect cleaning of a cip [5]

Figure 8 shows a sketch of the use of a bag for indirect cleaning of a chip inside an ultrasonic bath.

The tools required for this method are: ultrasonic bath, filled only with water up to the indicated filling height, cleaning liquid a tweezers, plastic bags, rod.

Using ultrasound for cleaning is a common practice in almost all laboratories around the world. Ultrasonic baths are used specially when mechanical brushing or other cleaning procedures are not possible; e.g. fragile structures or small dimensions in a microchannel which are difficult to reach.[5]

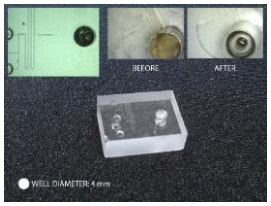

Fig.9 The result of cleaning a glass microfluidic chip [5]

Figure 9 shows the result of cleaning a glass microfluidic chip that contains a large reaction / analysis site and several inlets and channels.

Such a chip is difficult and not cheap to make; however the chip becomes unusable when the well is clogged with by-product of the reaction. Using ultrasonic cleaning inside a bag, the well can be emptied and the device reused..[5]

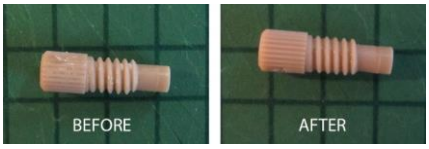

Fig. 10 Cleaning an Upchurch Connector .[5]

Figure 10 shows the cleaning of an upchurch connector can become contaminated and is difficult to clean due to the small grooves. Ultrasonic cleaning ensures rapid cleaning of the connectors. [5]

8. Conclusions

1. Various constructions of microfluidic circuits have been researched in order to perform blood tests with advantages that conventional technologies do not have.

2. Various concepts have been developed for the flow circuit of the microfluidic liquid and the components of the final device; Following a thorough analysis, a final version was chosen, which was verified through simulations in COMSOL Multiphysics and designed in 3D in the AUTODESK Inventor program.

3. A lab-on-a-chip (MEMS) microfluidic device was modeled for practical realization on a millimeter-sized silicon plate, using photochemical microtechnologies; the device is used for blood tests

4. This microfluidic device has major advantages, among which: it provides a fast result of this analysis; uses a very small volume of blood sample.

5. Following the analysis for transport, the distance traveled and the delivery time are reduced by 43% and 71%, respectively, with positive implications in reducing transport costs and human resources, while respecting the delivery conditions communicated and accepted by customers.

6. The outsourcing of the order delivery activity has positive effects in reducing the transport costs by approximately 20%, ensuring a greater capacity for their permanent adjustment, through regular price analysis, selection of new carriers, negotiation of new prices. Also, the delivery with external carriers eliminates the risk of syncopes in the delivery of orders, determined by the inability to use the car due to periods of parking for repairs or during the absence of the driver.Cost reductions may differ on each delivery route depending on the daily optimization taking into account route changes due to the information provided by the profile software regarding road traffic.

9. Bibliography

[1] Jingdong Chen, Di Che, (2013) „Progress of Microfluidics for Biology and Medicine”, Nano-Micro Lett. 5(1), 66-80 (2013)

[2]. Catherine McKenna, Efficient cleaning of a microfluidic chip, available at: https://blogs.rsc.org/chipsandtips/2016/02/08/efficient-cleaning-of-a-microfluidic-chip/?doing_wp_cron=1643008479.7458579540252685546875&fbclid=IwAR2JVEYci7OLn1Ee_vQFKGfVQCy1fJtyptE6Tr6SAesqY5R1A3qo4rWszcc, accessed on date: 28.02.2022

[3] Ghiculescu, D., Curs Ingineria Asistată a Micro și Nanotehnologiilor, available at: https://fiir.curs.pub.ro/2019/, accessed on date: 03.04.2022.

[4]*** „Fabrica microfluidica pentru auto-asamblarea asistata a nanosistemelor” available at: https://www.imt.ro/MICRONANOFAB/rez13.php accessed on date :29.03.2022

[5]*** ”Catherine McKenna, Efficient cleaning of a microfluidic chip”, available at: https://blogs.rsc.org/chipsandtips/2016/02/08/efficient-cleaning-of-a-microfluidic-chip/c, accessed on date: 03.05.2022