Novel Fabrication Method of a Microfluidic Continuous Flow PCR

Sina Jafari Ghalekohneh
University of Tehran
Mahdi Moghimi Zand (mmoghimizand@yahoo.com)
University of Tehran Faculty of Engineering https://orcid.org/0000-0002-7868-1438
Mohammad Dehghan Banadaki
University of Tehran
Babak Kamali Doust Azad
University of Tehran
Saba MirAhsani
Amirkabir University of Technology

Research

Keywords: , Microfluidic, DNA Amplification, Continuous Flow, Temperature Cycle

Posted Date: May 19th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-28384/v1

Abstract

In this study, simulation, design, and fabrication of a Micro PCR based on MEMS and LoC are done. The designed PCR has 2 temperature zones. In fact, in this PCR, the second and third stages of the experiment are merged inside one temperature zone. In the first step, a proper design based on genetic studies is considered. Then, to find the location of the heaters, the system is numerically studied via COMSOL Multiphysics. The designed PCR in this study has 2 temperature zones of 95 and 60 centigrade. After that, the heat transfer analysis is validated. The dimensions of the chip are 80 mm in length and width and 7 mm in height. There are 64 channels on the chip which means the PCR has 32 cycles. The length of each microchannel is 33 mm and each temperature cycle is 68 mm. The width of the microchannels is 500 mm and the depth of it is 400 μm (following Mohr et al. study (30)). Different methods for fabrication of the microchannel has been used, which in the end, laser engraving was chosen. Isopropyl Alcohol (IPA) is used for the bonding. Also, the thickness of the cap is considered 2 mm to strengthen the chip. Heater and Peltier (thermoelectric cooler) is used to reach the desired temperatures. The reason to use the Peltier is that the 95 centigrade zone can affect the 60 centigrade zone which increases the temperature at this zone. A PID controller is used to control the temperatures. The main advantage of this research is the very fast, easy, and low-cost method for producing PCR chips. It can be useful in critical situations, such as COVID-19 pandemic conditions where there is an urgent need for PCR chips to diagnose this disease.

Introduction

MEMS devices that use light and fluid mechanics have introduced a new era for MEMS technology. Microfluidics is related to the technology of fluid mechanics in the micro or nanoscale. Microfluidic technology is a new technology that has been able to utilize the special properties of micro and nanoliter fluids, as well as reducing the cost and time of testing, and has gained wide applications in biomedical and medical researches. A microfluidic device is a chip made of silicon, glass, or elastomer in which micron-sized tubes are embedded and fluids flow through the tubes or channels. Considering the many advantages of microfluidic systems and their high flexibility to produce new structures, it is necessary to introduce and develop this technology in the diagnosis and medical laboratories. With the development of micropumps, fluid flow microsensors, micro-valves, and ... gradually the main purpose of microfluidics, which is the biological and chemical sciences, became apparent. The important thing about microfluidics is that as the fluid-dominated space becomes smaller, new features of the fluid can be analyzed. Low fluid content is the key point of microfluidics. In such a way that this small amount in small dimensions causes changes in fluid behavior. Besides, when the sample volume is very low, the number of particles in it will be greatly reduced, making it much easier to analyze.

Microfluidic systems have a wide range of applications. Because there are many research and diagnostic experiments in the field of biology and medicine in which samples and soluble materials are tested, a wide range of applications of such systems are in these areas. As each part of the chip has a function equal to that of a laboratory. Therefore, this technology is called "Lab-on-a-chip".

Great advances have been made in the biological sciences since the discovery of the two-stranded DNA structure by Watson and Carrick (1) 66 years ago. These advances have led to the emergence of branches of science such as modern biotechnology and genetic engineering. PCR has played a major role in the evolution of the life sciences, altering the attitude of scientists in medical research, basic sciences, criminal trials, and environmental research. PCR is a sensitive method for detecting the desired DNA even in very small amounts. PCR has many benefits that have expanded its use in laboratories. Speed, simplicity, reasonable price, and an increased variety of related products are the advantages of PCR. It is interesting to note about the importance of PCR that Mullis won the Nobel Prize for this invention eight years after the invention of PCR.

PCR Steps

In general, PCR is performed in three separate steps. These three stages are separated due to temperature differences:

1)The DNA template is first trimmed to separate complementary strands.
2) xThe reaction is at a temperature where the oligonucleotide primers can bind to the template DNA.
3) The reaction is heated to a temperature close to the optimum temperature of the polymerase activity.

The number of copies of the target DNA is doubled per cycle if the PCR efficiency is 100%. After these 3 steps, the first cycle is complete, the next cycles are exactly like the first cycle. In this way, the DNA fragment increases exponentially after multiple cycles of PCR. As a result, after 20 cycles, the target gene will be over 250,000. Therefore, PCR is an efficient method for amplifying a piece of DNA. Also, these three thermal zones can be merged to two thermal zones. in this study, a two-temperature PCR chip was investigated.

Applications of this device include making multiple copies of a particular gene, prenatal diagnosis, fetal sex determination (2), definitive diagnosis of the disease, proving the identity of the suspect on hair samples in criminal cases, etc.

The polymerase chain reactions used for DNA replication to analyze gene function in diseases were invented in 1984 by Carrie Mullis (3). This simple chemical process had a huge impact on the medical, biological and diagnostic issues (4) (5) (6) (7)

The first microfluidic PCR was reported in 1993 (8). Since then, microfluidic PCRs have attracted the attention of scientists because of their importance in the biological sciences. Also, the cost of fabricating the device has dropped dramatically, which can be extremely beneficial given the limited resources available.

Traditional PCRs were performed in such a way that the whole system and the compartment, as well as the PCR solution, were heated and cooled together. So, the amount of energy needed to warm and cool the complex was high. This also prolonged the PCR reactions (between one and two hours). However,

recent research has shown that very little time is needed for both denaturation and annealing to allow the sample to reach equilibrium temperature (9). Therefore, the time required for denaturation and annealing steps, which was high in traditional PCR, can now be dramatically reduced (between 20 and 30 seconds). Traditional PCRs also had high reagent consumption and their amplified gene content was much lower than their reagent consumption.

Sample preparation and post-PCR analysis had to be done offline, which made it difficult to integrate PCR on a lab-on-chip system.

These problems can be solved by the recently developed microfluidic technologies. Micro-PCR, for example, can transfer heat rapidly due to its high surface-to-volume ratio, and also accelerates the mixing due to the propagation effect as well as the small size of the microchannels. On the other hand, sample management, detection, blending, and separation can be done on a single chip. Besides, the thermal cycle time is dramatically reduced. Due to the rapid response of the sample to the surroundings of the PCR solution, it has a uniform temperature during the test and thus improves the performance of the device. An attractive feature of small PCRs is their portability, which makes it easy to diagnose and analyze disease locally. However, contact between surface and reagent/sample and high surface-to-volume ratio result in restriction of PCR and transmission of contamination which are two of the most important microfluidic problems. These problems can be solved by the introduction of micro-droplet technology. The PCR solution appears in microdroplets, eliminating surface contact and reagent/sample and also preventing contamination transmission.

Currently, micro-PCR devices are classified into two groups: Well-based micro-PCR chips, for example [(8) and (10) (11) (12) (13)] and continuous-flow micro-PCR chips (14) (15) (16) (17) (18) (19).

Well-based Micro-PCR Chips

In PCR-based wells, the PCR solution is injected into a well and then the whole set, containing the solution is warmed and cooled to the desired temperature cycle. As a result, well-based PCRs require a large amount of energy, which produces unwanted thermal inertia effects. These effects increase the duration of the test.

Continuous-Flow Micro-PCR Chips

Continuous-flow micro-PCR drives the fluid in predetermined thermal regions to reach the desired temperature cycle. This type of chip has less thermal inertia because only the PCR solution needs to be warmed and cooled and the whole set should not be heated or cooled. This makes the heat cycle faster and consumes less energy. It also provides the system for portability applications as well as integrating the entire PCR system on a chip. Continuous-flow micro-PCRs have different designs. For example, oscillatory devices (20) (21) (22), Closed-Loop devices (23) (24) (25), and Fixed-Loop devices [(15), (26) (27) (28) (29)].

As mentioned, the type of device intended for design and fabricate is a Fixed-Loop continuous flow PCR, due to its lower cost, faster time to obtain results, and less energy waste. To fabricate this chip, the number of microchannels must be predetermined, and then after studying all the fabricating methods and choosing the appropriate method, the fabrication of the device starts.

Design And Simulation

In this study, the results of Mohr et al. (30) research were used and the dimensions of the microchannels are following the dimensions of that research. COMSOL Multiphysics was used to simulate the system and check the temperature of each part. The mesh used in this simulation is normal and simulated at several different temperature states.

A PCR requires two temperatures of 95 ° C and 60 ° C for optimal results. The channels were designed with a width of 500 µm and a depth of 400 µm and a length of 33 mm for each channel. The volume of solution that enters the device per minute is 60 microliters, resulting in a 5 mm/s inlet velocity. The inlet rate can be changed according to the type of flow and PCR test. Figure 1 shows an outline of the system.

And the two temperature zones created are shown in Fig. 2. Considering the type of PCR test and the desired thermal zones, the position of heaters can be modified.

As previously mentioned, two PCR temperature zones (95 and 60 ° C) are required for a PCR cycle. In figure (3), the simulation is performed with COMSOL when only the 95 °C heater is turned on.

The 95 ° heater is positioned at the right half of the system and the left half is connected to open air. As shown in figure (3), in many areas of the left half, the temperature is affected by the 95 ° heater and the temperature is above 60 ° C which is above our optimum temperature. So, to reach the desired temperature zones that are 95 degrees in the right half and 60 degrees in the left half, we have to somehow reduce the temperature at the left half part. Therefore, a Peltier is installed on the left heater to cool down the temperature to 60 degrees Celsius.

In Fig. 5, the comparison between the simulated chip in this study and Mohr et al. research is provided.

Materials And Methods

Fabrication of microchannels using micro-milling

Creating microchannels using micro-CNCs and micro-milling is an alternative approach in the micro-industry that is less discussed. Micro-milling is a fabrication method that creates microchannels using cutting and discharging raw materials. Micro-milling is a low-cost manufacturing process that uses rotary cutting tools to remove materials from a piece. Recent studies have shown that micro-milling is an effective manufacturing method in the field of microfluidics. Micro-machined tools have also been used to make the connection between oil and water to isolate DNA, RNA, and protein. But it also has many

disadvantages that must be taken into account when choosing a fabricating method, such as the high cost of the equipment, the need for a large area to place the device, and the need for unique technical expertise. However, recent advances in machining technology have mitigated many of these drawbacks, and micro-milling became an important method in microfluidics.

In this study, the overall system was first sketched in CATIA (figure (6)) and then transferred to the CNC machine, shown in figure (7). Then microchannels were fabricated by CNC. The carved piece is shown in figure (8).

It should be noted that as shown in Fig. 8, the channel inlet and outlet were initially placed on the system surface. To create the inlet, the Plexi surface had to be punctured by drilling, causing the Plexi to crack and break. To solve this problem, we created the inputs and outputs as connected to the channels, sketched in Fig. 6.

The disadvantages of this method include the retention of additional swarf in the microchannels, which blocks the channels. Also, the cost of the piece was much higher than the laser method.

laser engraving to create microchannels

Laser engraving, which is a subset of laser marking, is the use of the laser to engrave an object. This method does not use ink or drill tools that have to contact the surface, and this is an advantage to this method, as in other methods the tool or ink should be replaced regularly but this problem does not exist in this method.

A laser device consists of three main parts: the laser, the controller, and the studied surface. The laser acts like a pencil. The radiation emitted by it allows the controller to follow the pattern on the surface. The controller direction, the speed of movement, and propagation of the laser beam on the surface are targeted. The type of surface must be selected in such a way that the laser can affect it.

The point where the laser touches the surface should be on the focal plane of the laser optical system, which is often the focal point of the laser. This point is usually very small, perhaps smaller than a fraction of a millimeter (depending on the wavelength of light). When the laser passes over the surface, only the areas inside the focal point are largely affected by the laser. The energy transmitted by the laser changes the material on the surface at the focal point. The laser may heat the surface and thereby evaporate the material, or the material may break and flake on the surface.

Because the laser location is precisely analyzed by the controller, there is no need for a barrier to hold the object surface to prevent deviation from the designated engraving. This reason is primarily one of the reasons that distinguish this method from traditional engraving methods. The cost of using a laser is also significantly lower than the other methods. In our study, the next and final method used was laser engraving (Fig. 9). This method is as accurate as described above and is much less expensive than the micro-milling method.

At first, the CATIA file was given to the laser system and the power of the system was set to 22 watts to create a depth of 400 micrometers, which was the initial design of the system. The laser-engraved piece is illustrated in Fig. 10.

If we increase the power of the laser system, the Plexi surface will melt and the channels will be tilted. (Fig. 11)

After comparing the fabricating methods, we decided to choose laser engraving to continue this project.

The substrate material of the chip, PMMA

Poly (methyl methacrylate) with the chemical formula n (C5O2H8) is a chemical compound that is used as an intermediate polymer in the electron scanning scheme. It is one of the toughest polymers with higher transparency than glass and a polished, glossy surface resistant to atmospheric agents. Polymethyl methacrylate sheets, which also referred to as Plexi, have significant resistance to atmospheric and sunlight. They have excellent optical properties and a transparent surface while being more resistant to impact than glass. Besides, they have very low moisture absorption and good tensile and electrical resistance. About 40–50% of this polymer is used in the automotive industry, 33% in the construction and lighting industries, and the rest is used in the production and design of CDs and electrical industries.

Bonding microchannels

Polymeric fabrication methods generally create open microfluidic channels. Only in some specific cases, channel sealing is easily achieved. Open microchannels are used in systems that use capillary property, but the use of open channels is relatively limited. Therefore, the channel bonding should be made after the microchannel fabrication process. The choice of channel bonding method for polymeric microfluidic chips is as broad as that of microfluidic structure fabrication

Solvent bonding

In this method, the solvent that solves the polymer is applied to the polymer sheet and dissolves the top layer of polymer. Structural polymer sheets and cover sheets are pressed together and the sheets bond to each other as a result of solvent evaporation. The advantage of this method is that we do not need high temperatures for bonding and also there is no need for expensive tools except in cases that alignment is needed.

Solvent selection and adhesion time for each type of polymer should be optimally selected. The low temperature in this method makes it theoretically possible for film deposition of biologically sensitive materials. One of the disadvantages of this method is that the solvent readily reshapes the structure, especially if there are smaller structures that need to be bonded. At worst, the entire channel can be blocked by the dissolved polymer. To reduce these problems, the solvent use of time should be reduced.

After creating the microchannels on the surface, the set must be prepared for the channel bonding operation. For this purpose, the engraved piece is first placed in an ultrasonic device for one hour to get clean. The ultrasonic device vibrates to remove extra swarf in the channels and cleans the surface of the channels. (Fig. 12)

After cleaning the lids and the channels, it is the time to bond them. Before that, we put the microchannels under the microscope. Figure 13 shows the image of the microchannels under the microscope.

Chloroform

First, Chloroform was used to bond. By soaking the cotton with chloroform and rubbing it to the surface of the lid and the channels, we quickly press the two together. However, this method was not suitable because of chloroform high dissolving power. As can be seen in Fig. 14, it dissolved much of the channel wall and the channels became distorted and some of them were blocked.

IPA

The next method was using IPA (Isopropyl Alcohol) to seal the channels. In this method, as before, the cover and substrate were placed in an ultrasonic machine for one hour and then spray the IPA solution onto the surface of the channels as well as the cover. Then we put them on each other and pressed together. It should be noted that the pressure must be distributed equally all over the surface, otherwise due to the high concentration of pressure in specific places of the surface, the cover, and the channels will be distorted (Fig. 15)

To prevent this problem, 4 clamps were used and they were placed on the surfaces of the cover and the substrate symmetrically. Besides, to distribute the pressure equally, two pieces of rubber were placed between clamps and the cover and substrate. (Fig. 16)

Then it was placed in the oven at 70 ° C for 30 minutes. It should be noted that if the duration of warming is increased, the channels will start to tilt and the channels may be blocked.

After the bonding operation, as shown in Fig. 17, the channels were well sealed and the water flowed as a test fluid through them and no leakage was observed.

Inlet and outlet flow paths creation

In many cases, the creation of an inlet flow path in polymer microchips is done by a drill or laser. In this way, the inlet and outlet flow paths are created using such devices before the microchannel is created on the polymer sheet. Note that drilling to create inlet and outlet routes must always be done before the bonding, as this requires high precision and control in drilling. Besides, the dust and particles left over by the drilling will easily block the channel.

Appliances used in thermal circuits

Temperature controller

In this study, KACON's PID Controller, KT Model was used to control the temperature of various temperature zones (Fig. 18). This controller reads the temperature from the sensor at any time, applying a suitable amp to the heater. The temperature measurement in this study is performed by a K-type thermocouple, which has a higher speed to sense temperature than other sensors and thermocouples.

Thermal elements

The heater used in this study is a customized 4 cm x 7 cm coil of electro-element shown in Fig. 19, manufactured by Abid Co. with input voltage 220V AC and 50 Hz.

Thermoelectric cooling

The Peltier used in this study is TEC1-12706, which has a nominal input voltage of 12 volts and can handle up to 15 volts. (Fig. 20)

8 1.5V batteries are used to turn on the palletizer. Then place it on the heater and set the heater temperature controlled by PID to 60 ° C.

Figure 21 shows the system after arranging the heaters and Peltier and placing them in the desired locations.

Inlet and outlet flow paths

We embedded the pipes into the inlet and outlet flow paths. Silicon adhesive is also used to seal the inlets, which are the joints of the pipes and the chip (Fig. 22).

Results

The fabricated system is 80 mm long, 80 mm wide, and 7 mm high. The height of the substrate and cover is 5 and 2 mm, respectively. This PCR has 64 microchannels (32 cycles) and the length of each channel is 33 mm and the length of a complete temperature cycle is 68 mm. (Based on Mohr et al. study (30))

To fabricate the chip, laser engraving method was used for creating the microchanenels. For bonding the microchannels, we used IPA (Isopropyl alcohol). For the temperature circuit, thermal elements and Peltier were used. Peltier was used for the situations that the temperature rises more than the desired temperature. PID controller was used for adjusting the temperature at various thermal zones.

In this study, the simulation, design, and fabrication of continuous flow PCR based on MEMS and lab on a chip was performed. The reasons for this choice are:

- Lower cost
- Faster analyzing of the sample
- Less energy wasting

Continuous-flow PCRs are divided into three categories: oscillatory, open-loop, and closed-loop. The type of continuous-flow PCR selected in this project is closed-loop continuous-PCR. This type of PCR is portable under any circumstances and does not require advanced laboratory or hospital environments. This feature can be very useful in deprived areas and places where there are no hospitals or clinics, such as war zones, and can accelerate patient care.

Conclusion

The main advantage of this research is the very fast, easy, and low-cost method for producing PCR chips. It can be useful in critical situations, such as COVID-19 pandemic conditions where there is an urgent need for PCR chips to diagnose this disease.

To conclude and as a recommendation for people who may need help in the future, these points would be useful:

- In this study, if the smallest dimension is greater than 200 μm laser engraving is best suited for the fabrication of microchannels, due to its appropriate accuracy and also a time-effective fabrication. It also costs much less than other methods. However, for different types of laser machines, the minimum engraving dimensions may be less or more than 200 μm.
- If the chip material is PMMA (Plexi), IPA can be used to bond the channels since its low cost and the speed of bonding using IPA is very convenient. There is also no need for advanced equipment.
- If IPA is used for sealing, because the chip should be placed in the oven for a while, the ratio of the cover thickness and the substrate be at least 0.4. If this ratio is too low and the cover thickness is much less than the substrate, then the cover and the substrate will be separated after some time, probably due to the difference in the thermal strain of them.
- To avoid the contamination of the samples, the droplet technology would be very useful. The samples would flow as droplets in a surrounded liquid which is often oil. By using droplets, the potential effects of channel surface roughness on the samples would be eliminated. Also using droplets can reduce the energy waste and the volume of the samples and also increase efficiency.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

All authors agreed to submit this study.

Availability of data and material

All data generated or analyzed in this study are provided in the article.

Competing interests

The authors have no competing interests

Funding

Not applicable.

Author’s contributions

Jafari Ghalekohneh, Moghimi Zand, and Dehghan Banadaki designed and wrote the article. Jafari Ghakohneh, Dehghan Banadaki, and Kamali Doust Azad carried out the fabrication. Jafari Ghalekohneh and MirAhsani conducted the simulation and design in COMSOL and CATIA.

Acknowledgments

We would like to thank MEMS/NEMS laboratory located in the School of Electrical and Computer Engineering, University of Tehran, Tehran, Iran.

References

1. *Molecular Structure of Nucleic Acids: A Structure for Deoxyribose Nucleic Acid.* **J**. **D**. **WATSON** & **F**. **H**. **C**. **CRICK**. 1953, Nature.
2. *Sex determination of human embryos using the polymerase chain reaction and confirmation by fluorescence in situ hybridization.* **Munné S**, **Tang YX**, **Grifo J**, **Rosenwaks Z**, Cohen J. 1994, Fertil Steril.
3. *A short history of the polymerase chain reaction.* **Bartlett JM**, **Stirling D**. 2003, Methods in Molecular Biology.
4. *Micro total analysis systems. 2. Analytical standard operations and applications.* **Auroux PA**, **Iossifidis D**, **Reyes DR**, **Manz A**. 2002, Analytical Chemistry.

5. *Miniaturised nucleic acid analysis.* **P**.-**A**. Auroux Y, Koc,**a A**. **deMello**, **A**. **Manz and P**. **J**. **R**. **Day**. 2004, Lab On Chip.
6. *Micro Total Analysis Systems. Recent Developments.* **Vilkner T**, Janasek D, Manz A. 2004, Analytical Chemistry.
7. *Total nucleic acid analysis integrated on microfluidic devices.* **Lin Chen**, **Andreas Manz and Philip J**. **R**. **Day**. 2007, Lab On Chip.
8. *DNA amplification with a microfabricated reaction chamber.* **Northrup MA**, **Ching MT**, **White RM**, **Watson RT**. 1993, Transducers.
9. *Rapid thermal cycling and PCR kinetics.* **G**.**Herrmann**, **Carl T**.**WittwerMark**. 1999, PCR Applications.
10. *PCR in a silicon microstructure.* **Wilding P**, **Shoffner MA**, **Kricka LJ**. 1994, Clin Chem.
11. *PCR detection of bacteria in seven minutes.* **P**. **Belgrader**, Benett W, Hadley D, Richards J, Stratton P, Mariella R, **F**. 1999, Science.
12. *Real-time nucleic acid sequence-based amplification in nanoliter volumes.* **Gulliksen A**, **Solli L**, **Karlsen F**, **Rogne H**, **Hovig E**, **Nordstrøm T**, **Sirevåg R**. 2004, Anal Chem.
13. *A simple device using magnetic transportation for droplet-based PCR.* **Ohashi T**, **Kuyama H**, **Hanafusa N**, **Togawa Y**. 2007, Biomed Microdevices.
14. *High speed polymerase chain reaction in constant flow.* **Nakano H**, Matsuda K, Yohda M, Nagamune T, Endo I, Yamane T. 1994, Biosci Biotechnol Biochem.
15. *Chemical amplification: continuous flow PCR on a chip.* **M**. **U**. **Kopp**, **A**. **J**. **de Mello**, **A**. **Manz**. 1998, Science.
16. *A closed-cycle capillary polymerase chain reaction machine.* **Sonin A**, **Ehrlich D**,**Chiou J**, Matsudaira P. 2001, Anal Chem.
17. *Plastic microfluidic chip for continuous-flow polymerase chain reaction: simulations and experiments.* **QingQing Cao**, **Min-Cheol Kim**, **and Catherine M**. **Klapperich**. 2011, Biotechnol.
18. Characterization *and analysis of real-time capillary convective PCR toward commercialization.* **Xianbo Qiu**, **Shiyin Zhang**, **Lanju Mei**, **Di Wu**, **Qi Guo**, **Ke Li**, **Shengxiang Ge**, **Xiangzhong Ye**, **Ningshao Xia**, **and Michael G**. **Mauk**. 2017, Biomicrofluidics.
19. *Advance in Research of Microfluidic Polymerase Chain Reaction Chip.* **Qi-DiHEDan-PingHUANGGuanHUANGZuan-GuangCHEN**. 2016, Chinese Journal of Analytical Chemistry.
20. Design *and theoretical evaluation of a novel microfluidic device to be used for PCR.* **M**. **Q**. **Bu**, **M**. **Tracy**, **G**. **Ensell**, **J**. **S**. **Wilkinson**, **and A**. **G**. **R**. **Evans**. 2003, Micromech.
21. *Quantitative study of the adsorption of PCR reagents during on-chip bi-directional shunting PCR.* **P**.**A**. **Auroux**, **P**.**J**.**R**. **Day**, **A**. Manz. 2005. Proceedings of the 9th International Conference.
22. Direct HE *Pyrrolidine Sulfonamide Catalyzed Michael Addition of Aldehydes to Nytrostyrenes.* **Wang**, Li W, Luo Z-X. R, **LU**, S-H, Xu, Yang A-D, **Y**.-**J**. 2005, Micromech. Microeng.
23. *A nanoliter rotary device for polymerase chain reaction.* **J**. **Liu**, **M**. **Enzelberger**, **S**. **Quake**. 2002, Electrophoresis.

24. *Application of magnetohydrodynamic actuation to continuous flow chemistry.* **West J**, **Karamata B**, **Lillis B**, **Gleeson JP**, **Alderman J**, **Collins JK**, **Lane W**, **Mathewson A**, **Berney H**. 2002, Lab Chip.
25. *Electrokinetic preconcentration and detection of neuropeptides at patterned graphene-modified electrodes in a nanochannel.* **BJ Sanghavi, W Varhue, JL Chávez, CF Chou, NS Swami**. 2003, Analytical chemistry.
26. *High speed polymerase chain reaction in constant flow.* **Nakano H**, Matsuda K, Yohda M, Nagamune T, Endo I, Yamane T. 1994, Biosci Biotechnol Biochem.
27. *Continuous flow thermal cycler microchip for DNA cycle sequencing.* **Wang H**, Chen J, Zhu L, Shadpour H, Hupert ML, Soper SA. 2006, Anal Chem.
28. *Microfluidic Continuous Flow Digital Loop-Mediated Isothermal Amplification.* **Tushar D**. **Rane**, **Liben Chen**, **Helena C**. **Zec**, **and Tza-Huei Wang**. 2016, Lab Chip.
29. *Loop-Mediated Isothermal Amplification on Crude DNA as a Point-of-Care Test for the Diagnosis of Mycoplasma-Related Vaginitis During Early Pregnancy.* **Yichao Wang**, **MD Bumei Zhang**, **BD Yan Sun**, **BD Yunde Liu**, **MD Yajun Gu**. 2017, Laboratory Medicine.
30. Numerical *and experimental study of a droplet-based PCR chip.* **S Mohr**, **YH Zhang**, **A Macaskill**, **PJR Day**,**R**. **W**. **BarberN**. **J**. **GoddardD**. **R**. **EmersonP**. **R**. **Fielden**. 2007, Microfluidics and Nanofluidics.

Figures

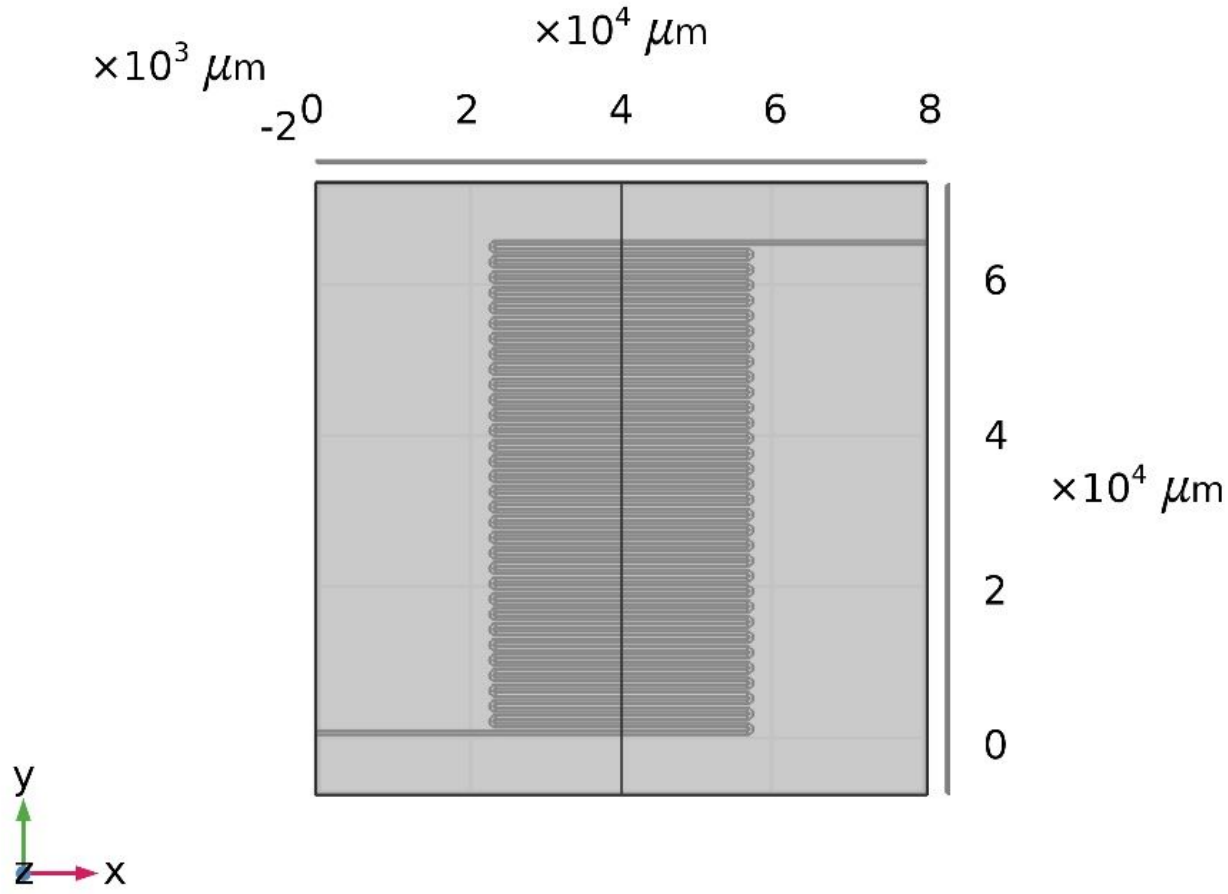

Figure 1

The outline of the system

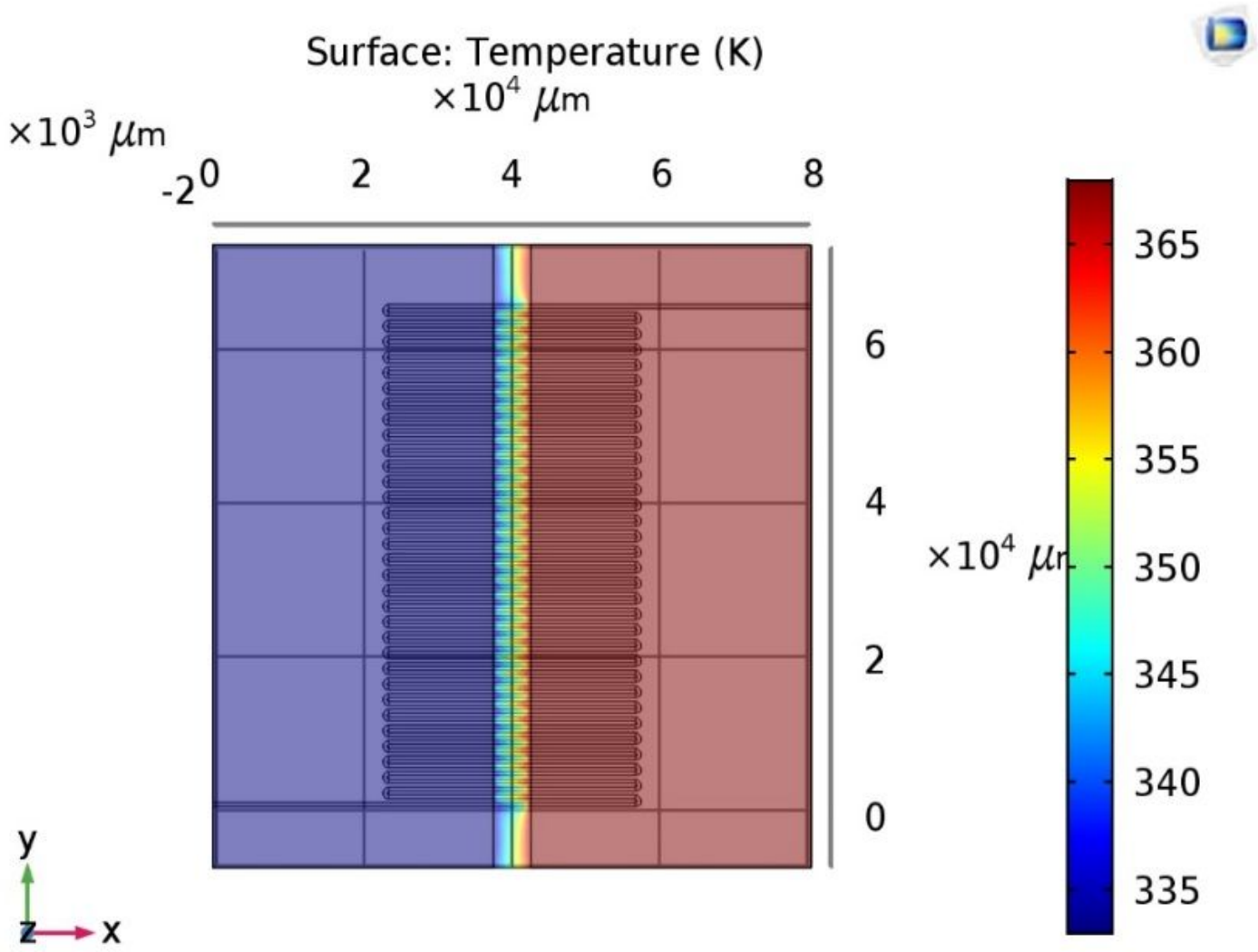

Figure 2

Temperature zones

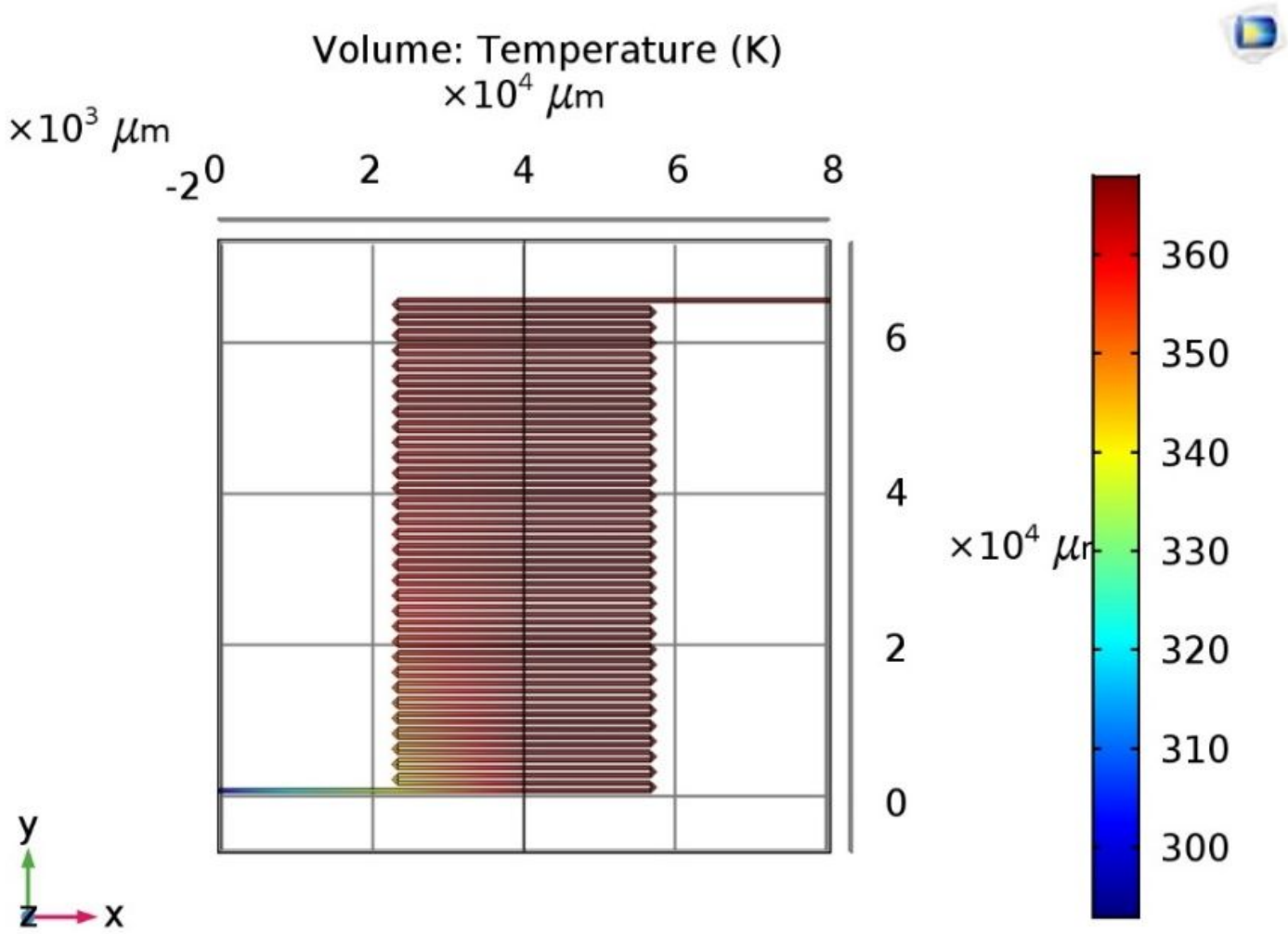

Figure 3

Solution temperature inside the channels when only 95 ° C heater is turned on

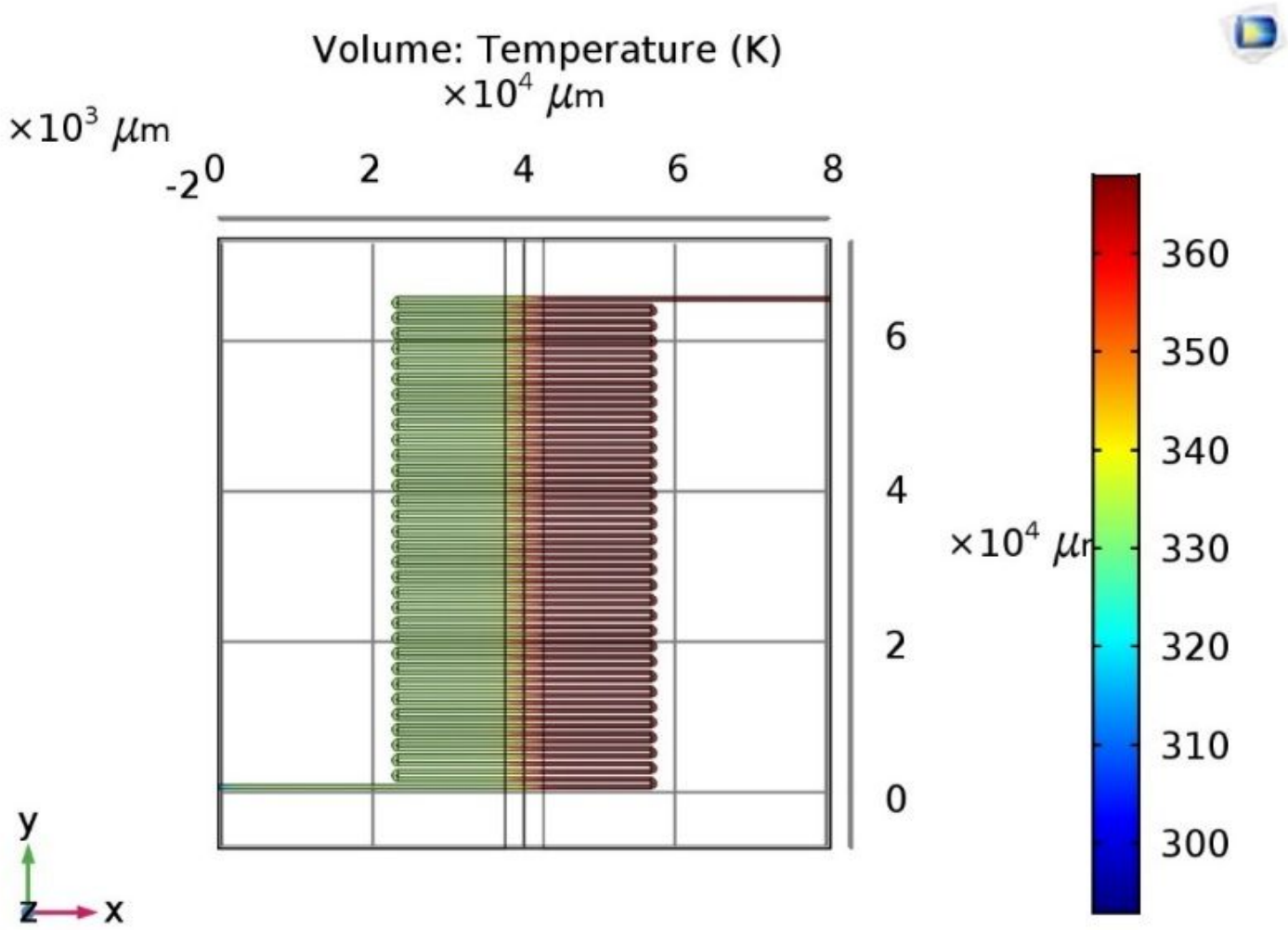

Figure 4

The solution temperature inside the channels when the heaters and Peltier are switched on simultaneously

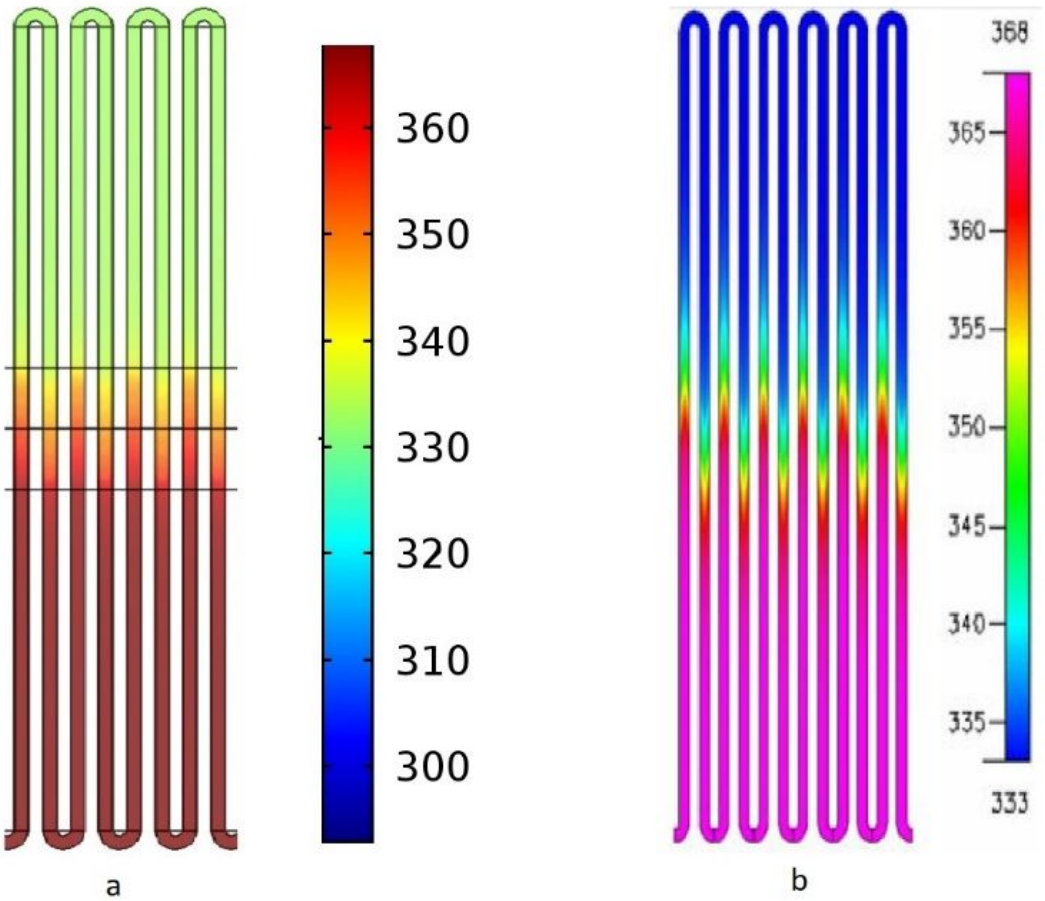

Figure 5

The temperature of the fluid inside the microchannels a) Present study b) Mohr et al. study

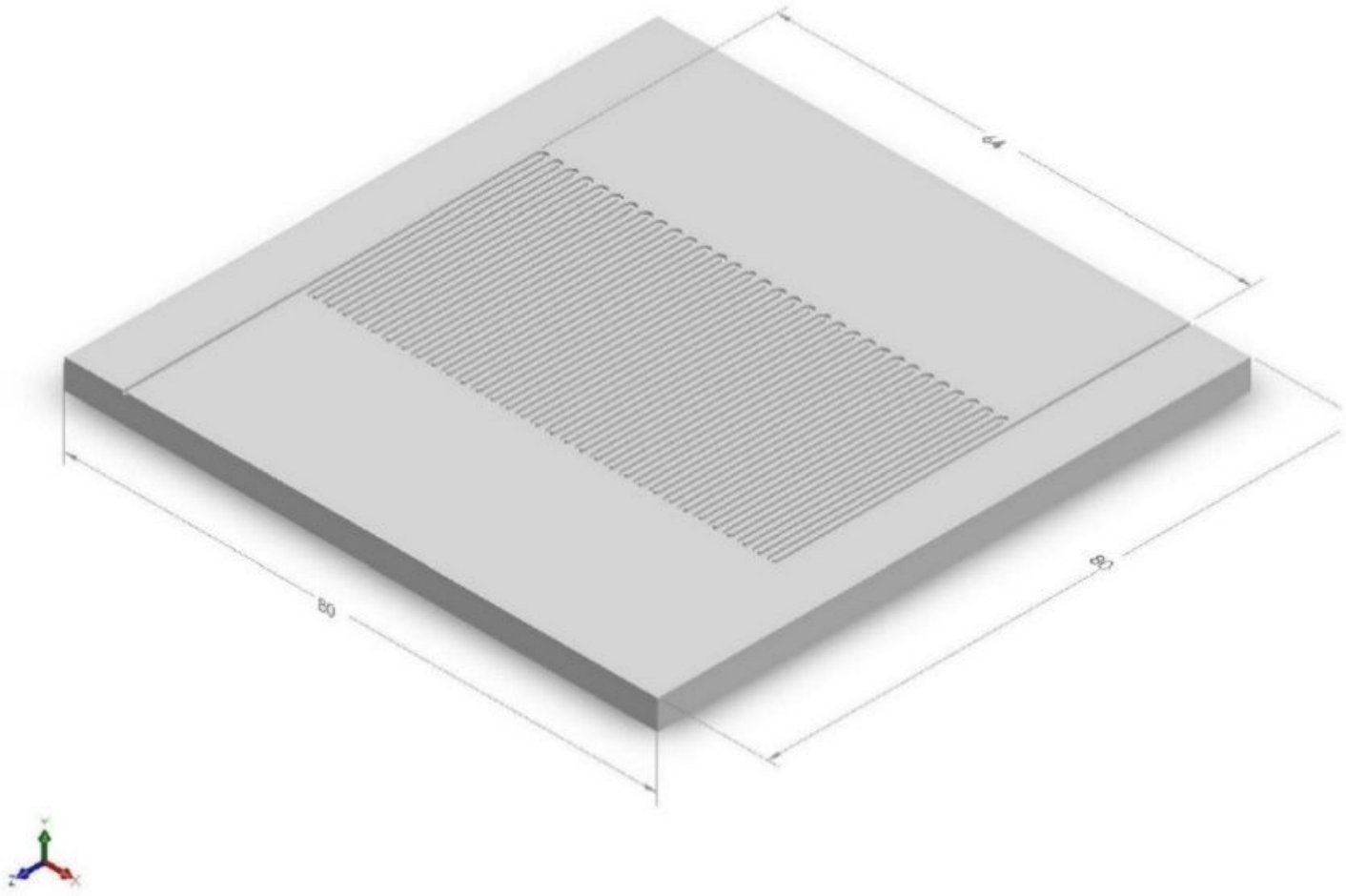

Figure 6

Outline of the system drawn in the CATIA software

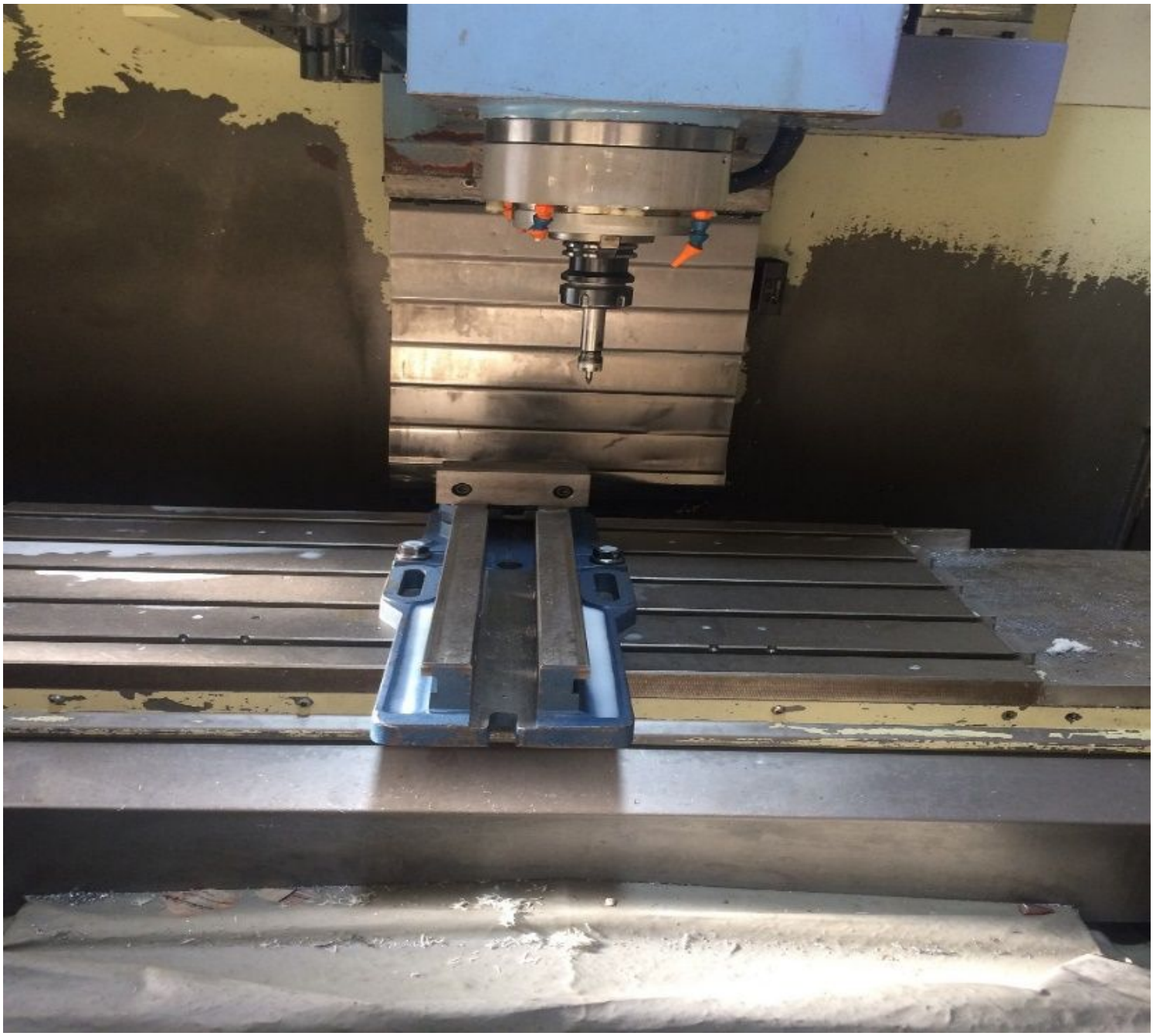

Figure 7

CNC machine used to create microchannels

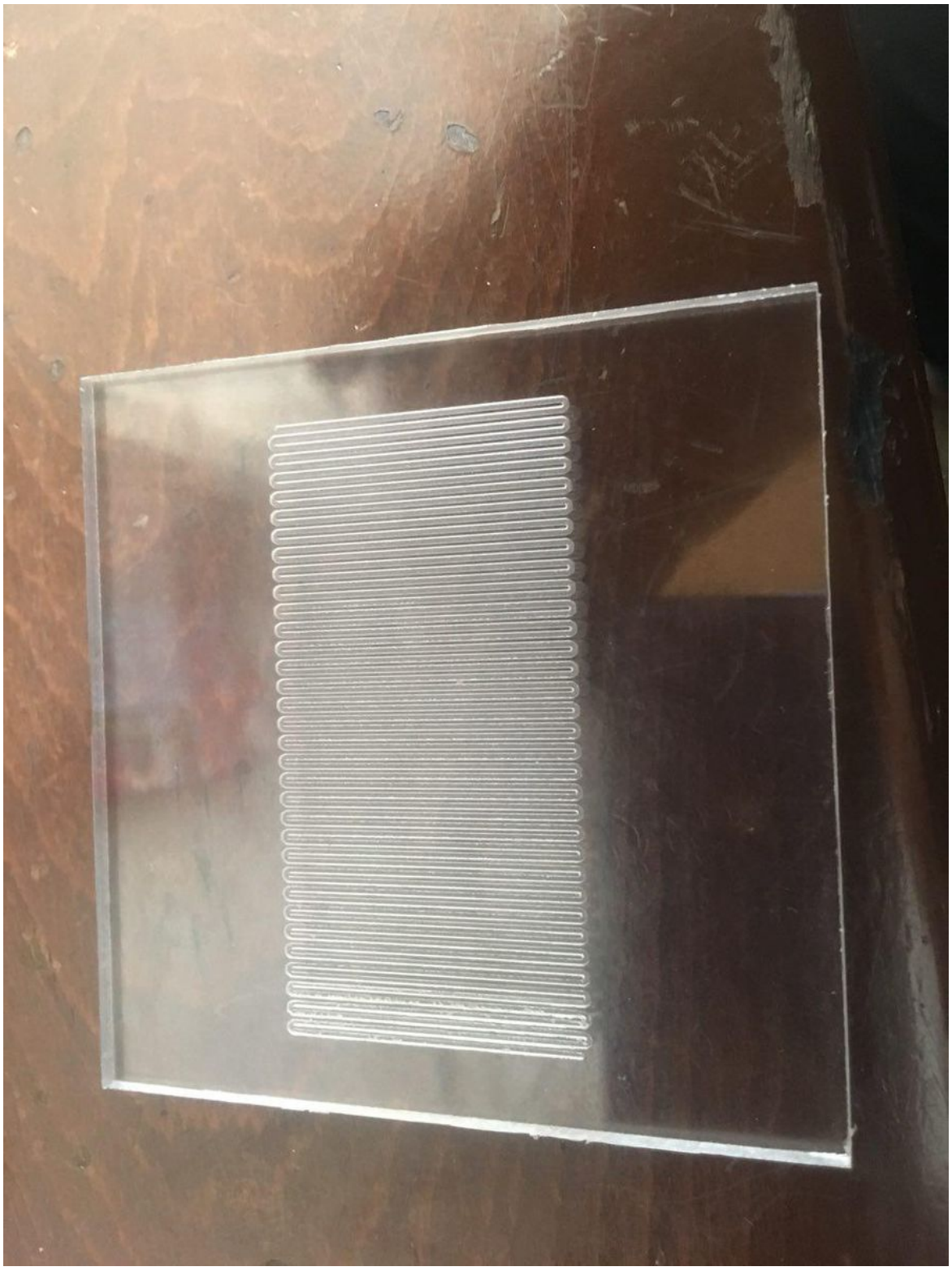

Figure 8

Microchannels created after machining

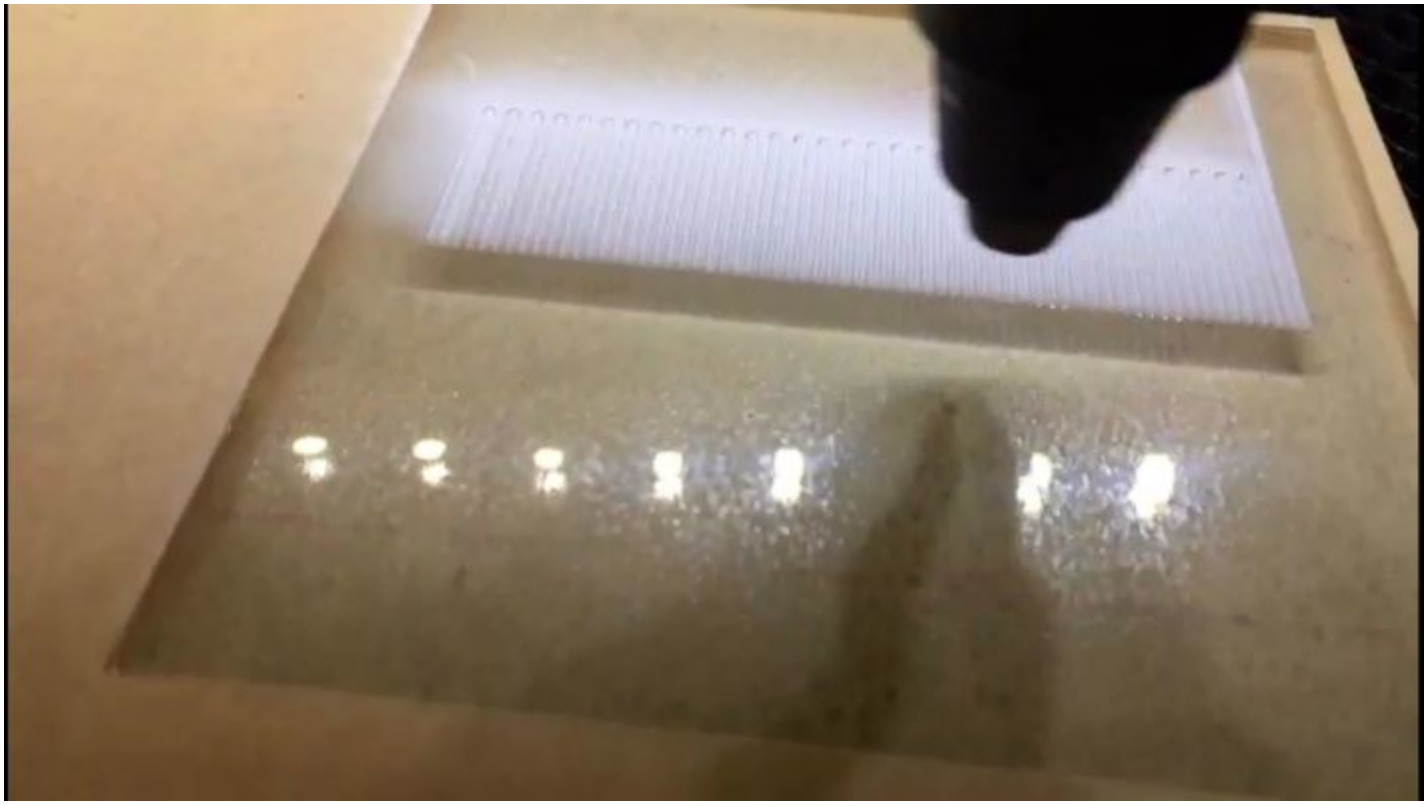

Figure 9

Creating microchannels using laser

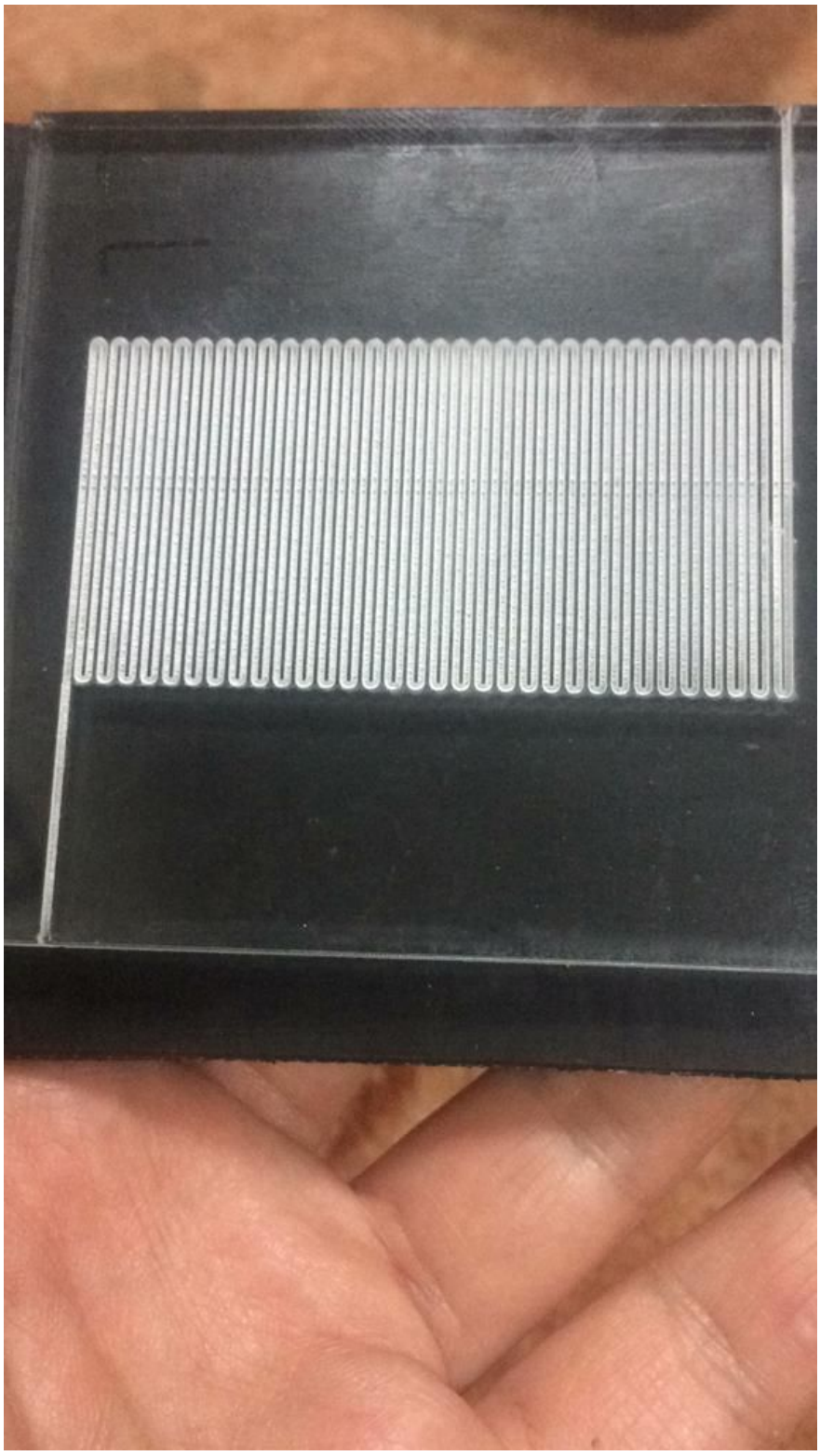

Figure 10

Laser generated design

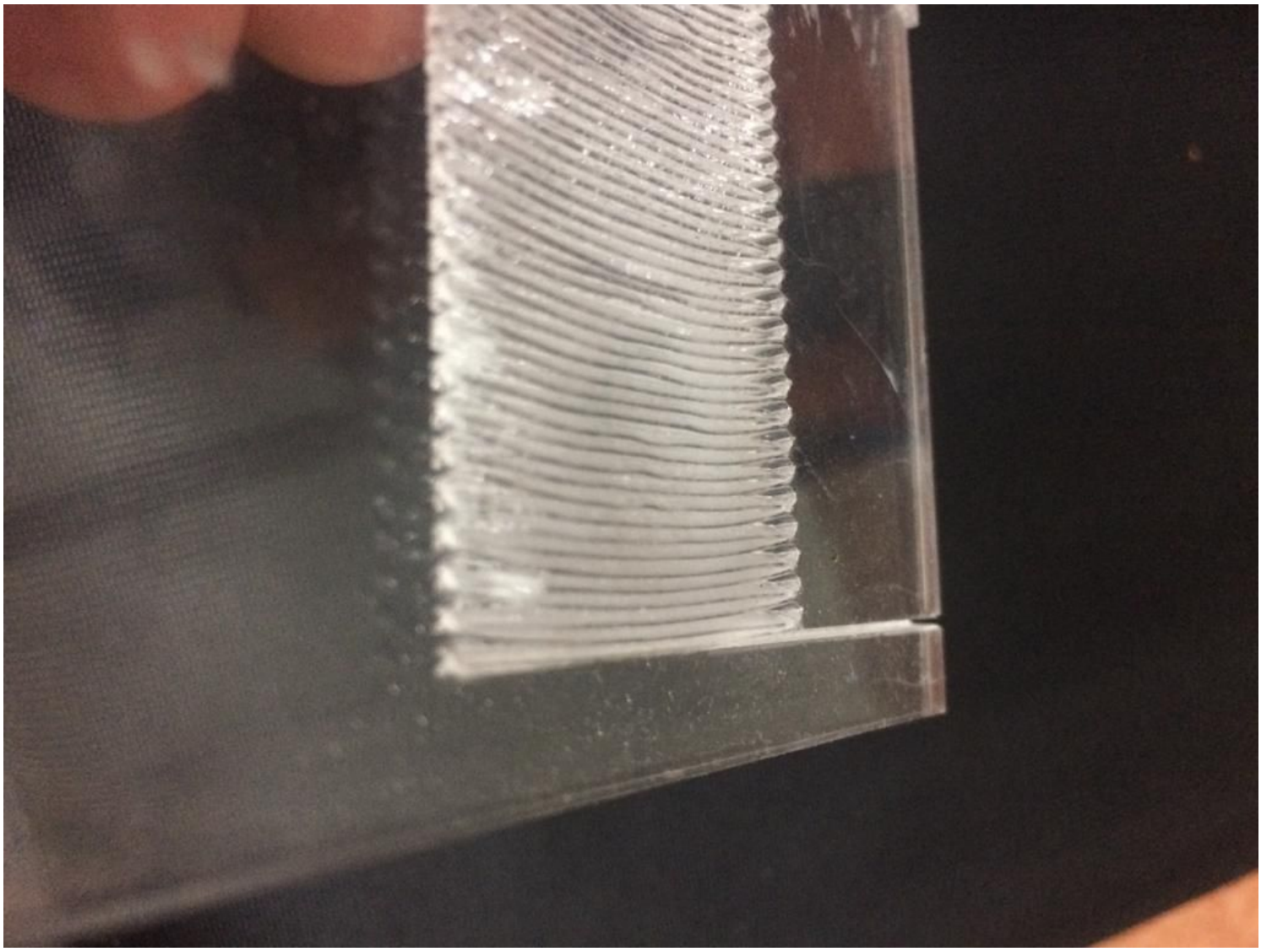

Figure 11

Distortion of the channels and melting of Plexi due to high laser power

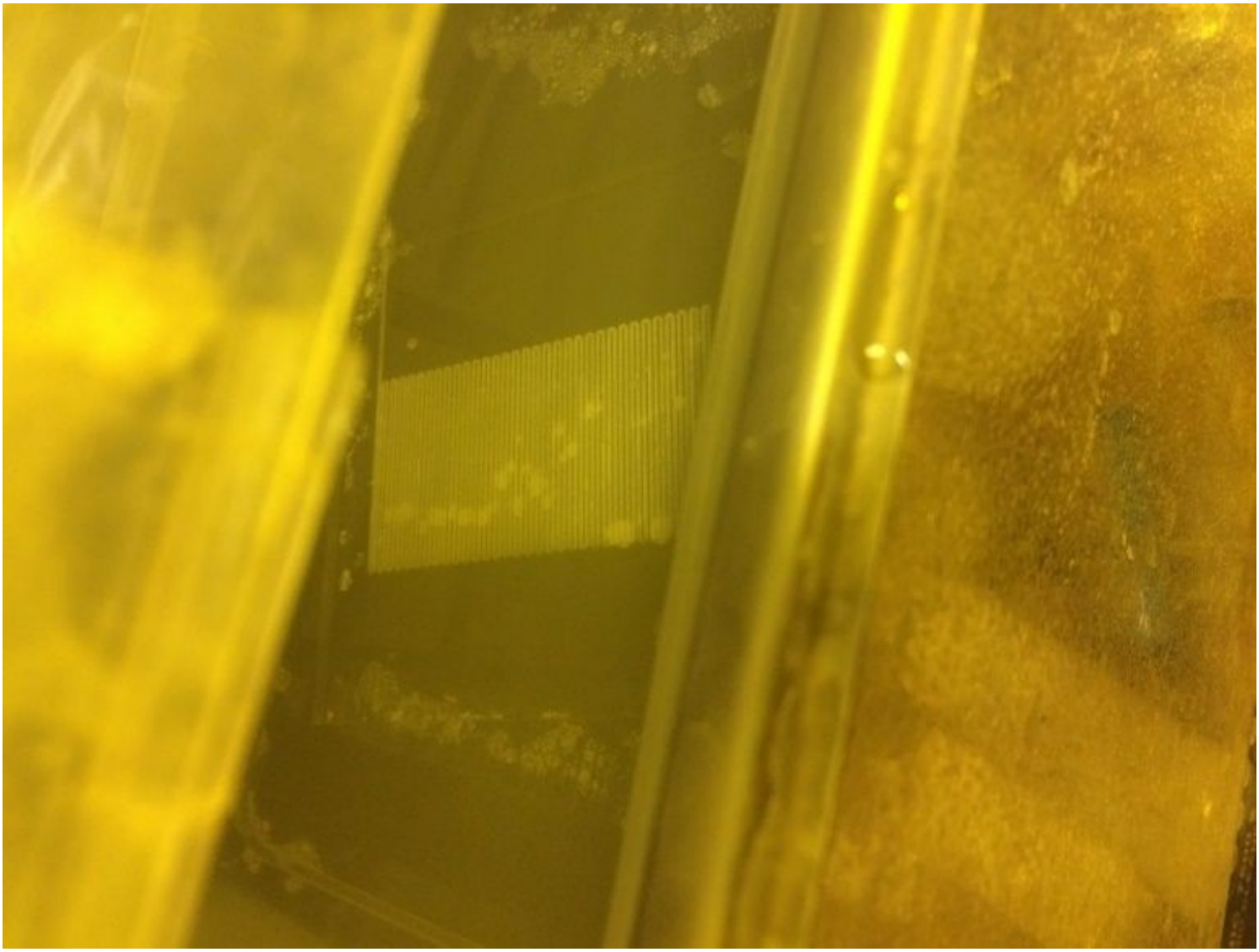

Figure 12

Chip cleaning via ultrasonic device

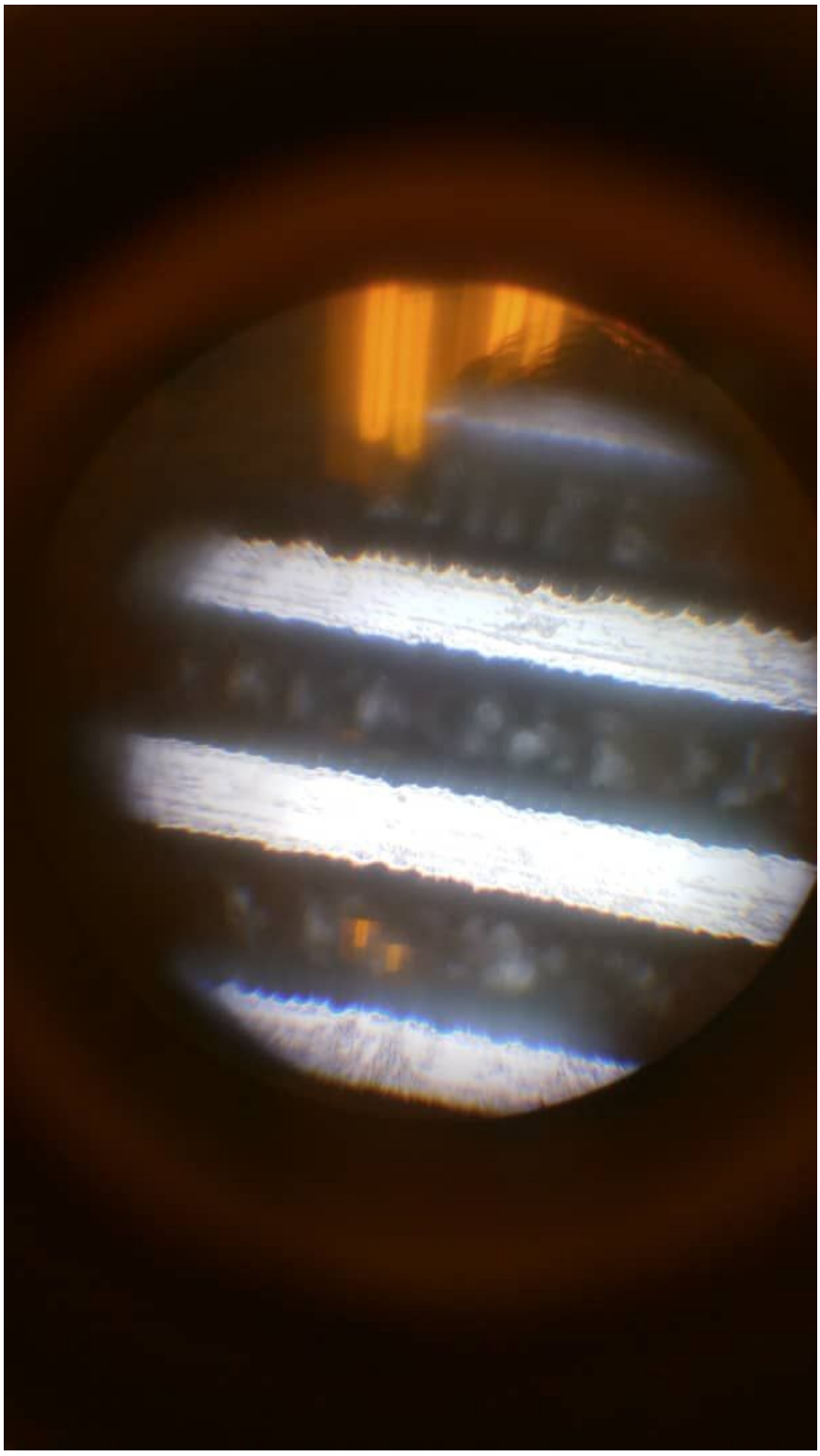

Figure 13

Microscopic image of the channel body

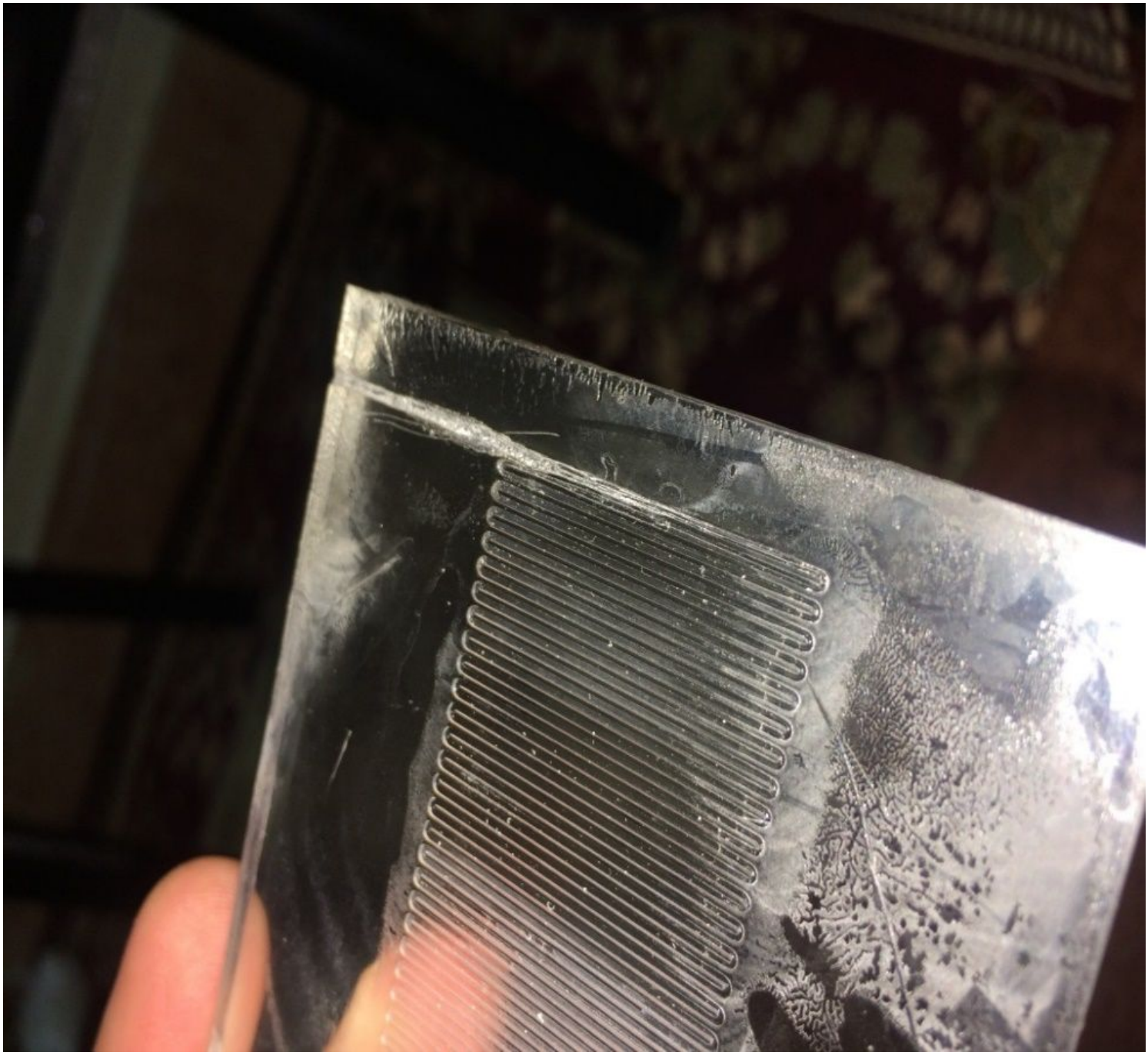

Figure 14

Channels are blocked because of the chloroform high dissolving power

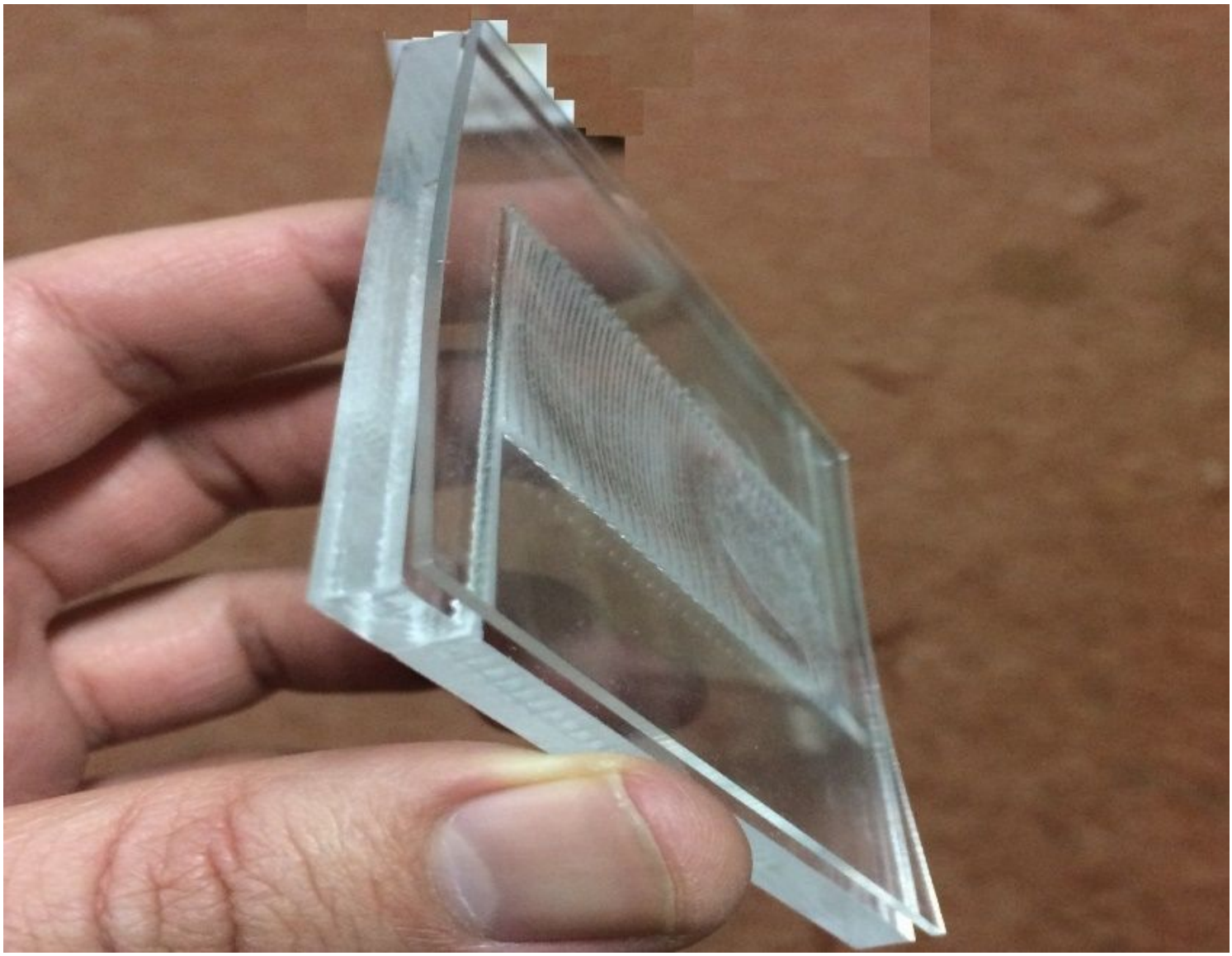

Figure 15

Distortion of the cover and microchannels due to asymmetric pressure concentration

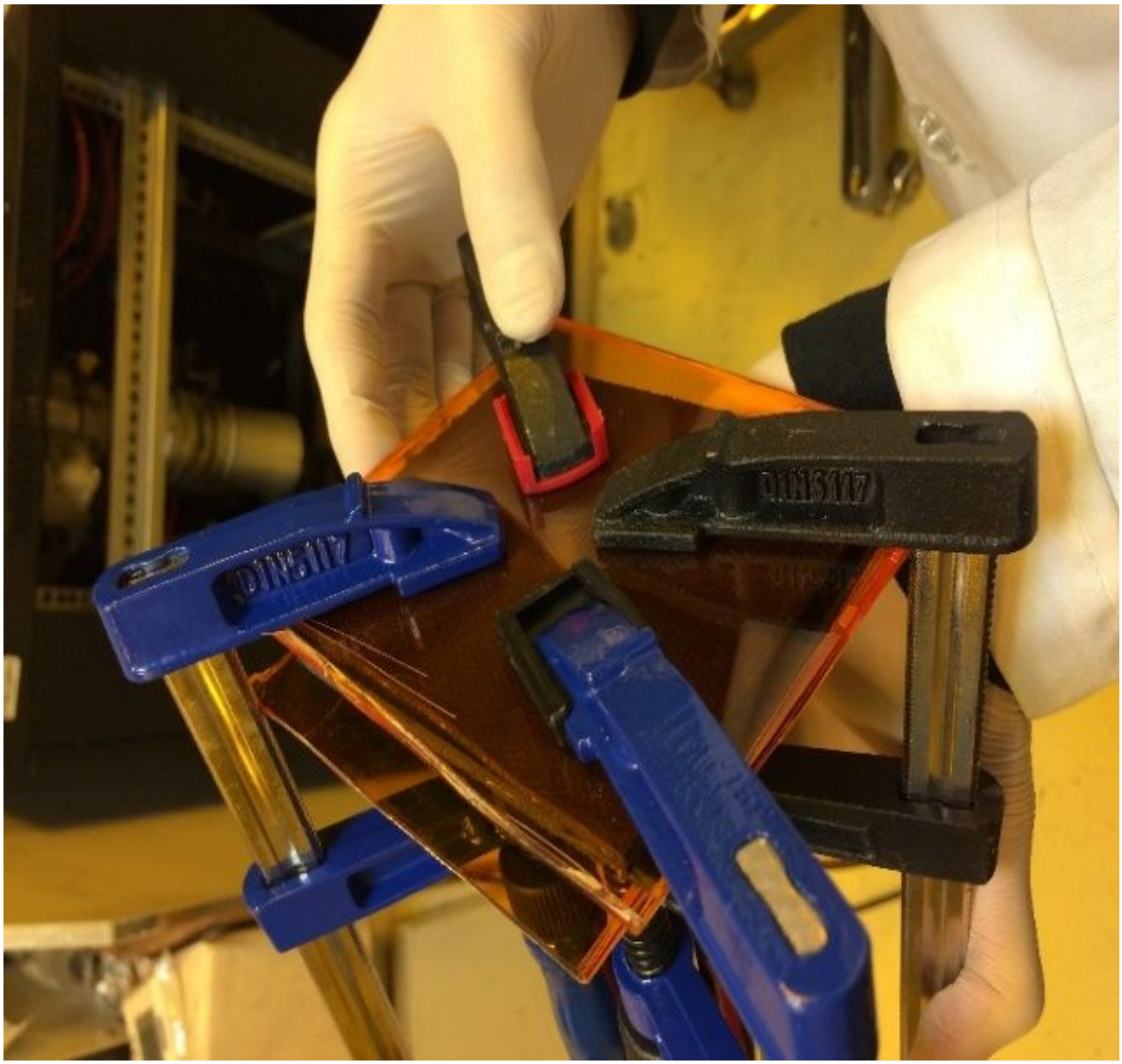

Figure 16

Using 4 clamps to symmetrically distribute pressure across the chip

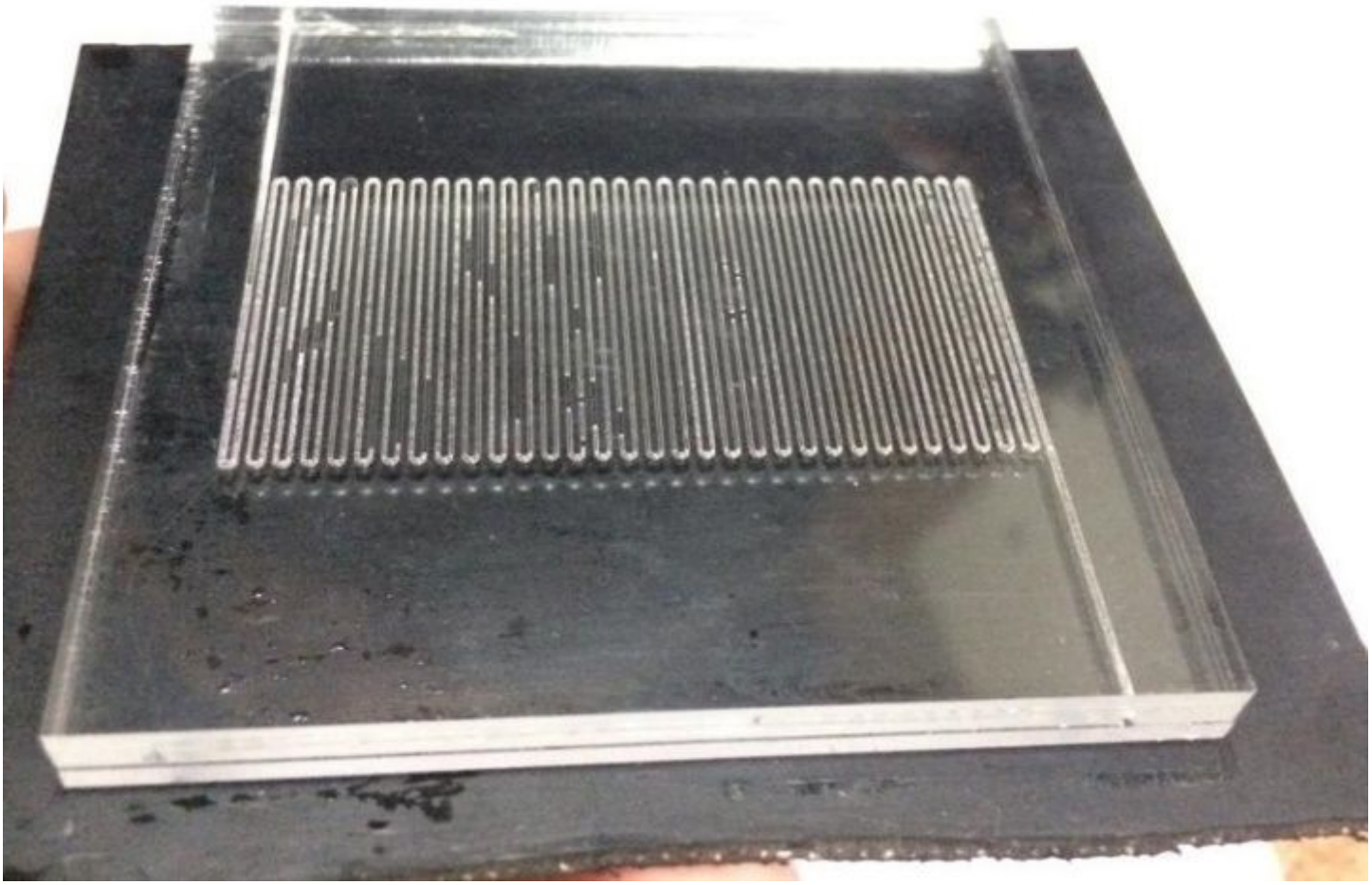

Figure 17

Chip and microchannels after bonding operation. Water was injected into the system in the form of droplets to ensure that the system was sealed

Figure 18

PID controller used in this study

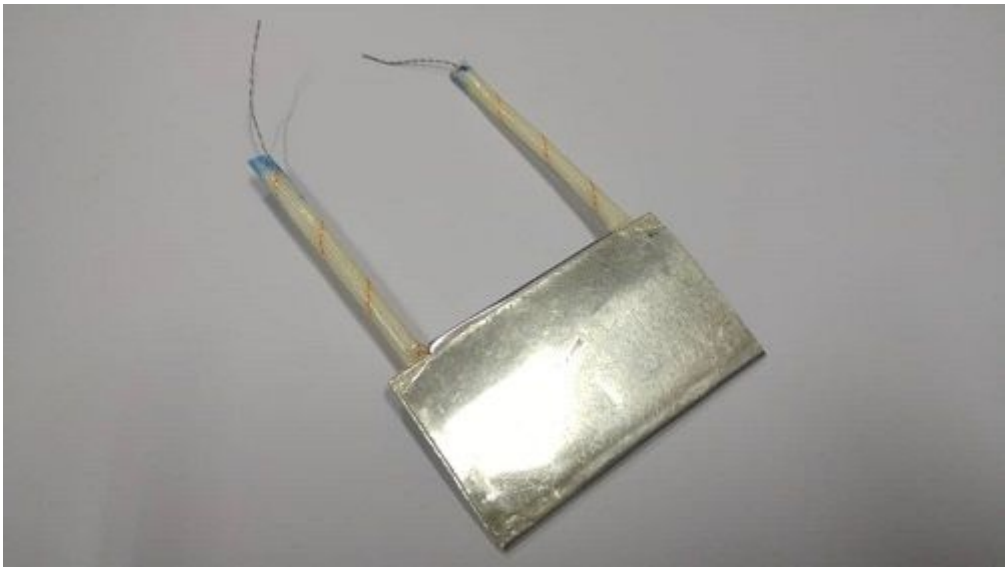

Figure 19

Customized 4cm x 7cm coil of electro-element

Figure 20

Peltier used in this study

a

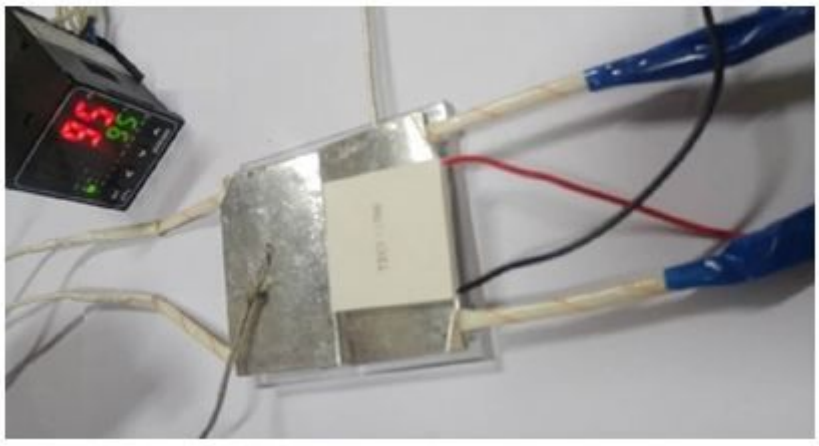

Figure 21

The temperature of the different thermal zones after turning on the heater and Peltier. a) 60 ° C b) 95 ° C

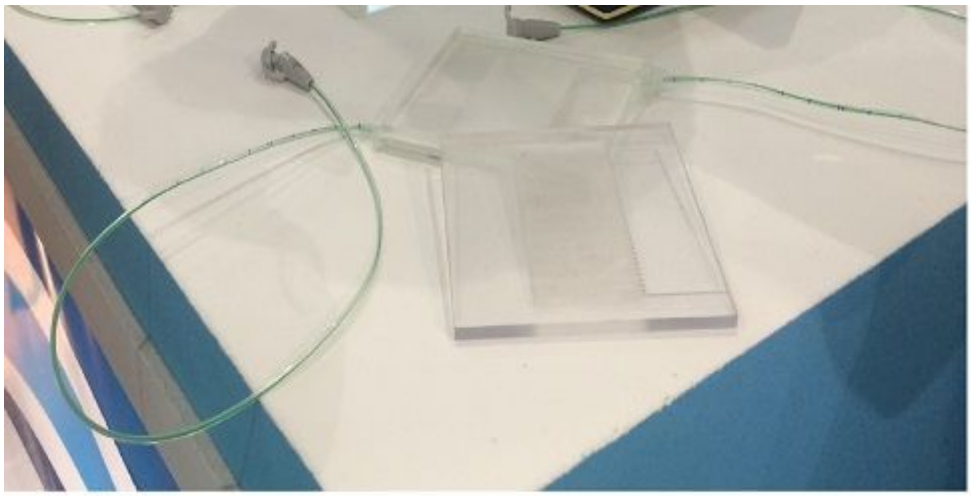

Figure 22

The inlet and outlet paths