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Master NANOTECH
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BlackHole Lab
172 rue de Charonne, 75011 Paris

DEVELOPMENT OF FAST AND SIMPLE FABRICATION PROCESSES FOR PAPER-BASED MICROFLUIDICS AND ALTERNATIVE POLYMERS TO PDMS.

STUDY OF A MICROFLUIDIC CHIP FOR AN AUTOTRANSFUSION PROCESS

from 2019/02/18 to 2019/08/18

Confidentiality: no

Under the supervision of:
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Present at the defense: yes

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Description of the company

The internship took place at BlackHole Lab, a startup in microfluidics founded in 2014 by a researcher. The start-up is part of a consortium of French companies working on microfluidics called "La vallée microfluidique".
BlackHole Lab is an equipment distributor for laboratories and companies to enable them to make microfluidic chips on their own. The company sells kits conceived to contain everything necessary to do a process and sold with a training, documentation written to explain the processes step by step and assistance if additional help is needed. All the processes are designed to be performed outside a clean room.

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Glossary

PDMS polydimethylsiloxane

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I. Introduction

Microfluidics is a scientific domain that deals with micro machined systems elaborated to manipulate and control liquids within channels that have characteristic dimensions in the range of micrometers to hundreds of micrometers (10^{-6} to 10^{-4} m) [1]. The Reynolds number corresponds to the ration of inertial versus viscous forces and is a quantity that determines the behaviour of a fluid. Under a critical value, about 2000, the fluid flow can be considered as laminar. The Reynolds number is defined by:

$$Re = \frac{\rho v L}{\mu}$$

with ρ the fluid density, v the fluid velocity, L a characteristic dimension of the channel and μ the dynamic viscosity.
Due to the dimensions of channels in microfluidic chips, the Reynolds number is always low enough for the flow to be laminar.

The dimensions of the channels also lead to many benefits: smaller amount of solvents and reagents are consumed leading to cheaper experiments, experiments are faster, changes of temperature can be done faster, several tests can be done on a single chip [2][3].

Microfluidics was first derived from microelectronics [4] which is why the first microfluidic chips were done on silicon wafers using microelectronic processes. Glass and polymers were later introduced and polydimethylsiloxane (PDMS) is currently one of the most used material in microfluidics [5]. Its main advantages are [6]:

- its transparency in the visible range that makes observation easier
- its biocompatibility that allows biological applications
- its permeability to gas and relative impermeability to liquids that makes it a material of choice for cell culture
- its ability to be covalently bonded to glass easily using plasma.

Microfluidics has applications in various areas such as medicine (diagnostics [7], lab-on-a-chip), environment (water treatment [8]) or biotechnology (organ-on-a-chip [9]). It is a field of study that has been raising interest increasingly over the past 20 years as shows Figure 1.

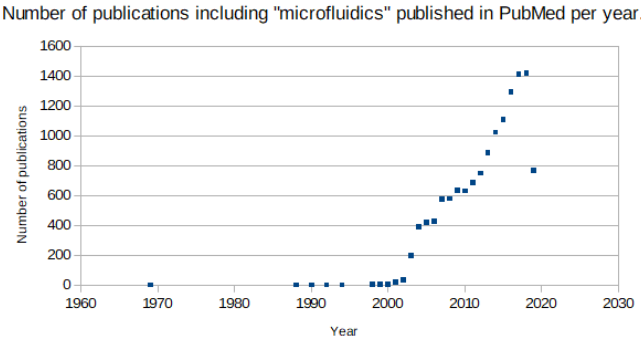

Figure 1: Evolution of the number of publication tackling "microfluidics" according to time, taken from PubMed website on the 24th of June, 2019.

In 2007, paper was introduced as a substrate for microfluidic chips fabrication. The goal was to find a cheaper alternative to polymers that would be processed more easily to make microfluidics more affordable and usable outside of a lab, for developing countries for example [10].

The initial objective of my internship was to develop a kit containing everything necessary to make **microfluidic chips on paper**. The kit would have to be complete, lead to a cheap fabrication process and an acceptable resolution (about 500µm is usually enough for paper-based microfluidics).
Once this kit elaborated, it was extended to two other applications: making the **molds necessary for the fabrication of PDMS chips** and the **development of chips made of polymeric films and an adhesive layer**.
Finally, I have worked on the design of a microfluidic chip that separates cells according to their size for an **autotransfusion system**.

II. Microfabrication kit development

The first part of the internship has consisted in developing a kit that was first aimed at paper-based microfluidics and then adjusted for other applications: the fabrication of molds for PDMS chips and the fabrication of microfluidic chips using adhesive and polymeric films. The development of this kit is detailed in this section.

II.1 Paper-based microfluidics

This first part of this section focuses on the fabrication of a kit aimed at paper-based microfluidics. First, paper-based microfluidics is introduced and the state of the art is detailed. Then the different methods of fabrication are explained and the choice of the equipment for the kit is detailed and characterized.

II.1.1 Operating principle

Paper-based microfluidics was first introduced by Martinez and Al. [10] from the Whitesides group in 2007. The idea was to design microfluidic devices with a simpler and less expensive method. The resulting device was aimed to be portable, easy to use and to dispose. The objective of this new approach was to make microfluidics affordable and usable without any laboratory or clean room for developing countries or to create an alternative to more expensive methods currently used in laboratories.

Unlike in conventional microfluidic chips, the fluid flow in paper is driven by capillary forces and controlled by the porosity and geometry of the device [11]. The size of the pores in paper being in the micrometre range, the Reynolds number remain small enough for the flow to be laminar. Therefore, the physical properties of the chips remain the same as in other microfluidic chips.

The porosity and thus the characteristic dimension is defined by the choice of paper and determines the speed of the flow while the geometry is defined by the fabrication process and determines the direction of the flow. The speed of the flow can be determined with Washburn's equation [12]:

$$L = \sqrt{D.t} = \sqrt{\frac{\gamma.r.t.cos(\phi)}{2\eta}}$$

with L the distance travelled by the fluid during a period of time t, D a diffusion coefficient, γ is the surface tension, r the pores radius, ϕ the contact angle between the fluid and paper and η the dynamic viscosity.

So the time needed for a liquid to travel a distance L is quadratic to the latter.

Paper is an hydrophilic substrate. In order to define flow path, channels are created by making hydrophobic barriers in paper. Several methods of fabrication are used to either change the shape of the substrate or its hydrophilic properties.

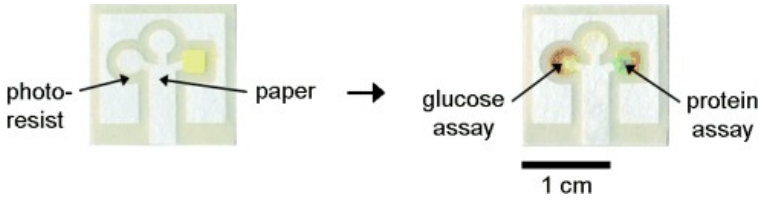

Figure 2: Picture of a paper-based microfluidic chip, taken from [10]

II.1.2 State of the art

The main advantages of paper-based microfluidics are that it is:

- **cheap and simple:** it varies from one process to the other but fabrication processes are usually cheaper, simpler and require fewer steps when paper is used as the substrate[13][14][15].
- **portable and robust:** one of the first reasons paper-based chips were designed was to be easily carried and used outside of a laboratory [10] to enable new applications including diagnostics in the field for developing countries.
- **easy to use:** the devices are usually straightforward, in one piece and most of the devices use colorimetric detection [16] which is easy to read and requires no equipment. Li and Al. [17] have designed a chip that determines the blood type and shows it in writing for a clear and easy interpretation of the result: the letters A,B or O as well as a plus or a minus sign appear according to the blood type (Figure 3). Some devices are designed so that the interpretation can be done by a smartphone application [18][19] and directly sent to doctors to make it easier for patients (Figure 4).
- **disposable:** paper being easily flammable, it is possible to dispose of the chips by incinerating them [20][21]. This represents a effective method of disposal requiring no specific facility.
- **biocompatible:** paper is biocompatible [22] and can therefore be used for biomedical and diagnostic applications.

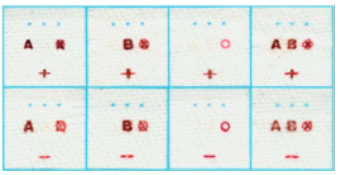

Figure 3: Results of a blood typing experiment [17]

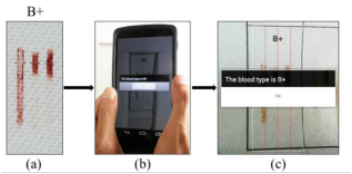

Figure 4: Interpretation of the results using a smartphone application [19]

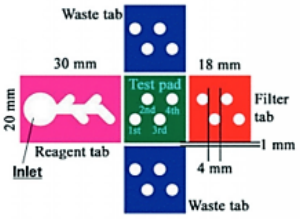

Figure 5: Origami-based microfluidic chip [23]

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Paper-based microfluidics is often used for its simplicity and low cost but also for the **new functions** it offers. It is possible to use the properties of paper to fold it and form 3D devices [23][24] (Figure 5), use solid reagents and a pen to carry and deposit it on paper by drawing [25]or use the paper for storage and immobilization of reagents without requiring specific surface treatment. Filtering can be achieved by the choice of an appropriate pores size and can be used to separate plasma from whole blood [26] for example.

The main **application** of paper-based microfluidics is diagnostics for ground operations in developing countries [10]. It can be to diagnose a disease [27] or to determine the presence or concentration of a particle in water [28]. The possibility to make the chips **outside a clean room** and with **limited and cheap materials and machines** as well as their **portability**, **robustness** and **mean of disposal** make paper-based microfluidics ideal for these application.

Yet, paper-based microfluidics has some limitations: it is necessarily single-use chips, some designs and applications cannot be done on paper and the resolution is usually around 500μm which is much higher than the resolution of PDMS features.

II.1.3 Existing methods of fabrication

There exists about ten fabrication methods for paper-based microfluidics, the main ones being:

- **Cutting:** the first envisioned method to control the propagation of a liquid in paper is to cut the latter to define channels where the liquid can propagate. The paper can be cut manually or automatically using a cutting plotter or a laser. Using a laser leads to more precise features, a resolution down to 25µm can be reached, but is also more expensive [29]. **Xurography**, the use of a cutting plotter to make designs [30], is a subcategory of this method.

- **wax-printing:** it is the technique the most widely used and discussed in the literature. Solid-ink printers, developed in 1986 by the company Tektronix, are printers using cartridges made of wax that is melted inside the printer and then deposited onto the paper. Wax being hydrophobic, such a printer can be used to print a design on paper [31]. The paper is then heated again on a hot-plate for the wax to melt and diffuse through the paper. An hydrophobic barrier is thus obtained in the paper substrate [32]. Unfortunately, this type of printer has been discontinued in 2018[33]. The main advantages of this technique are its simplicity and rapidity. The main drawbacks are that it is not suited for high temperature applications and requires an heating step.

- **Inkjet printing:** it is a second method using a printer. The printer can be used to dispense an hydrophobic solution like alkyl ketene dimer on the paper to locally change its hydrophilicity. But, it is also possible to first make the paper entirely hydrophobic and then use the printer to make it locally hydrophilic. Usually, the

sheet of paper is first dipped into a polystyrene solution and dried for two hours at room temperature to make it hydrophobic. Then, toluene is dispensed to create hydrophilic channels [34]. This technique can be done with a standard desktop printer [35] or a microdrop dispenser. It can have a good resolution and excellent reproducibility but requires the use of possibly harmful solvents.

- **Photololithography:** this process can also be used on paper [16] but, despite a higher resolution, it is not interesting because it is expensive, more complicated and requires a mask so we would loose the main benefits of paper-based microfluidics.
- **Other methods using a mask:** some methods like wet etching or the use of plasma are also possible but require a mask which complicates the process, makes it more expensive and less suited for prototyping.

II.1.4 Objectives of the kit

The objectives for this kit are:

- to have a kit that is **complete** : it must contain everything that is necessary during the chips fabrication as well as documentation to guide the consumers for the main processes.
- to have a good enough **resolution** for paper-based microfluidics. The average channel width is 500um in the literature.
- the process has to be **simple**, **cheap** and **fast**: these are some of this technologies' strongest advantages and they cannot be compromised during the elaboration of this kit.

II.1.5 Equipment choice and characterization

With all these objectives in mind, xurography is the fabrication method that fits the best: the cheapest machines cost under 200€ and their resolution is around 0.5mm which could be enough.

A cutting machine could prove itself useful in paper-based microfluidics for a fast prototyping. Although the resolution and accuracy may not be as high as the ones obtained with other methods, this would represent a simple, fast and cheap solution.

I had two models of cutting machines at my disposal: Portrait 2 from Silhouette, the desktop model of the brand, and Explore Air 2 from Cricut, a middle range machine. The two machines will be tested and compared in the following paragraphs to determine if they can be used to fabricate microfluidic chips and which one is the best suited for it.

H

	Cricut	Silhouette
Appearance	looks more professional tools holder and two compartments for tools storage	smaller and lighter noisier (blade's height reconfigured before each new project and it is noisy)
Ease of use	less intuitive but not so complicated	
Software	online, requires internet easier to access from any computer mobile app more intuitive/complete	on computer, available offline faster to load designs
Cutting parameters	pressure number of cutting cycles scroll wheel with preset and tunable parameters	blade's height, speed, pressure number of cycles
writing	simultaneous use of 2 tools (writing+cuting)	
cutting test	slightly better	tears materials more easily

Table 1: Comparison between the Cricut and Silhouette machines

Comparison tests: In order to compare the two machines, I have taken various parameters into account, from the appearance and easiness of use to the accuracy. The results and observations are gathered in Table 1.

In order to do the cutting test mentioned in Table 1, the design shown in Figure 6, made with CleWin 5, was used to determined the smallest feature that could be cut properly as well as the reproducibility and accuracy of the machines. The test has been performed on 90g/m² paper. The radius of the circles are 100µm, 200µm, 300µm, 500µm, 1000µm and the largest one is 5000µm with a spacing of 300µm, 400µm, 500µm and 1000µm respectively. Likewise the channels are 100µm, 200µm, 300µm, 500µm, 1000µm thick and 0.5cm long and the largest one is 5000µm thick and 1cm long.

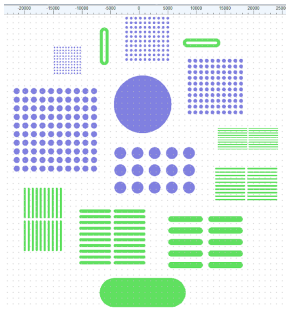

Figure 6: Design used to test the resolution and precision of the cutting machines, made with Clewin5

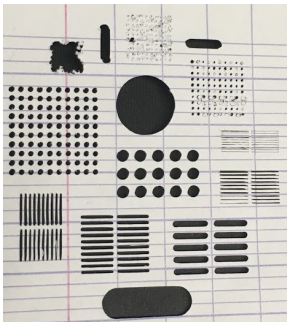

Figure 7: Picture of the results of the test made with the Cricut machine

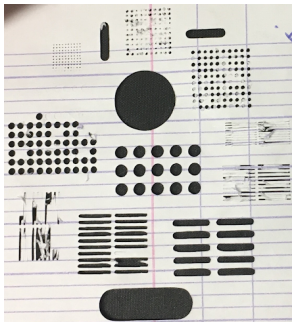

Figure 8: Picture of the results of the test made with the Silhouette machine

The figures 7 and 8 show the results using either the Cricut or Silhouette cutting machine. With the Cricut one, the channels are well cut down to 200µm thick, with less than 10% error margin and the circles are well cut down to a radius of 500µm. They remain reasonably well cut with a radius of 300µm: 73% of the circles were detachable.
With the Silhouette machine, the resolution is comparable for circles with 69% of the ones with a radius of 300µm detachable from the paper but it is worse for the channels: the smallest ones detachable are 500µm thick with a reproducibility of 90%. All in all the difference in cutting paper for these two machines is rather slim and performances are significantly dependant on the chosen parameters.

The Silhouette cutting machine has more cutting parameters that can be tuned and one that seemed interesting was the cutting speed. Yet, I was unable to successfully cut Whatman paper grade 4 with this machine. The paper is torn no matter the speed chosen. More generally, increasing the speed tend to tear the material.

Conclusion: The main arguments to make a choice between the two cutting machines are a better ease of use of both the machine and software of the Silhouette cutting plotter, the possibility to properly cut more substrates with the Cricut one and the possibility to use two tools at once for the latter. This feature could be used to create holding structures around a pattern that could be hydrophobized using a permanent marker for example (Figure 12). This is mainly for these last arguments that the **Cricut cutting plotter was chosen**.

II.1.6 Hydrophobic ink

Another method that has been explored is the use of hydrophobic ink: as the ink wicks through paper, it creates an hydrophobic barrier, thus delimiting channels. Some commercialized permanent markers contain hydrophobic ink and can therefore be used for this purpose [36]. Several markers have been tested for that purpose: to do that, it is possible to draw a circle with the permanent marker and add a droplet a water or another solution

at the center of the circle to see if it spreads outside of the circle. The effect of the ink is immediate but it may be necessary to draw the circle several times for the ink to fully penetrate the paper.
Some permanent markers contain a water-resistant ink that is not hydrophobic: water cannot be contained within a circle or a channel and will spread through the ink, without dragging it.

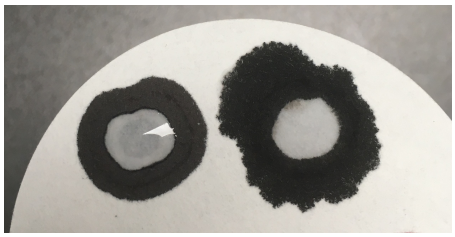

Figure 9: Picture of a drop of water in a circle of hydrophobic ink (left) and hydrophilic ink (right)

It is possible to combine cutting and the use of a pen with hydrophic ink. The design and supporting features can be cut and hydrophobic ink can be deposited on the supporting features (II.1.7).

II.1.7 Proof of concept

The first chip made with the kit to simply check its operation for a regular paper-based mirofluidic chip was a channel shaped in the letters "BHL" (Figure 10.1) in which purple ink was inserted. The channels are either one or two millimeters wide. On Figure 10.2, one can see that the blue pigments flow faster in the paper than the red ones. They must be smaller than the red ones.

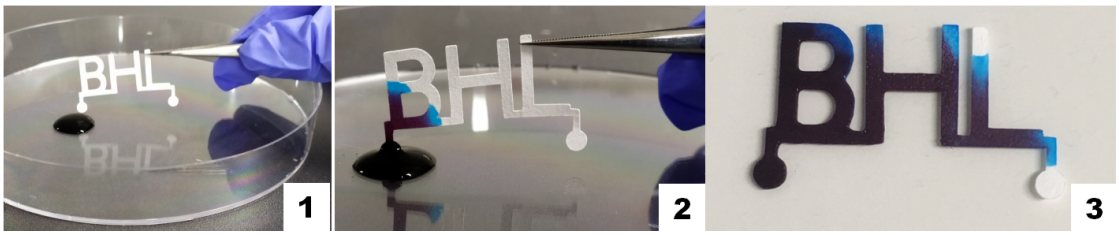

Figure 10: Pictures of the steps of the experiment done on a paper cihp

In order to test the simultaneous use of a blade and a pen, the design shown on Figure 11 was used and the result is shown on Figure 12. The ink has to be deposited after the cutting step otherwise the paper tend to tear itself. Yet, it is not easy to align the writing step and the cutting one so either the writing step can be done by hand or the design should include larger writing features and a first chip should be used for calibration.

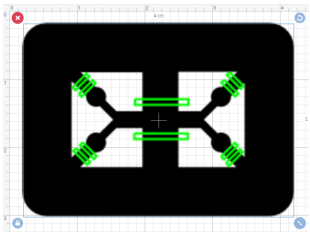

Figure 11: Design for the chip made by cutting and writing

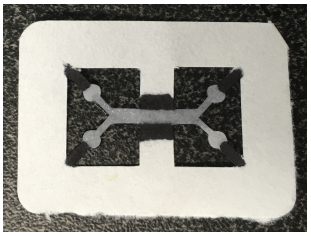

Figure 12: Chip made be xeurgraphy and hydrophobic ink for the holding features

II.1.8 Conclusion

In conclusion, I was able to put together a complete kit that respects all the objectives set at the beginning of the internship and listed in the section II.1.4. This kit offers a solution for a fast, cheap and simple process to make paper-based microfluidic chips with a resolution of 300µm.

While I was elaborating this kit, two other applications for it had been mentioned: using it to make molds for PDMS chips and to make chips based on adhesive films. These two extensions of the kit will be detailed in the following paragraphs.

II.2 Molds fabrication for PDMS chips

As mentioned in the introduction of this report, PDMS is the most widely used polymer in microfluidics. Indeed, this polymer has many upsides for this application: it is transparent to visible light, biocompatible and permeable to gases but not to liquids which allows cell culture in PDMS chips, it can be processed with a resolution down to the nanometer range [37] and easily stuck to a glass slide to close the channels using plasma.

In order to make PDMS chips, a mold is required and this section of the report describes the adaptation of the kit developed in the previous section for the fabrication of molds.

II.2.1 PDMS chips fabrication process

In this first subsection, the fabrication process of PDMS chips will be detailed.

First, a mold has to be made. The mold can be bought from a foundry or directly made in a clean room or a lab. There exists different solutions according to the resolution needed and the most used ones are:

- **Epoxy resin SU-8:** it is one of the most used material for molds in microfluidics thanks to its high resolution but it is also one of the most expensive ones [38]. SU-8 is a negative photoresist that is spin-coated on a substrate and then exposed according to a standard photolithography process.
- **Dry films:** photosensitive dry films like ordyl can be deposited on a substrate (glass) and laminated. The heat and pressure of the lamination step cause the film to adhere to the substrate. Once again, a photolithography process can be followed to obtain the features in the film.

Then, the PDMS chips can be fabricated. Here is the fabrication process for the PDMS chips:

Materials:

1. PDMS (liquid)
2. reticulation agent
3. a mold
4. plastic cup and glass rod
5. a pipette
6. a weighing scale
7. a vacuum chamber
8. an oven
9. a cutting tool
10. a petri dish
11. a plasma chamber
12. a glass slide

Protocol:

1. Place the plastic cup on the weighing scale and poor PDMS in the cup, 30g for example (Figure 13.1).
2. Use the pipette to add the reticulation agent in the cup so as to obtain a ratio of 10:1 (so 3g in this example) (Figure 13.2).
3. Mix energetically the content of the plastic cup with the glass rod (Figure 13.3).
4. Place the cup in the vacuum chamber until no more air bubble is present in the PDMS (about half an hour) (Figure 13.4).
5. Place the mold inside the petri dish, or any large enough container, and poor the PDMS on top of the mold while being careful not to create any air bubbles (Figure 13.5).
6. Place the petri dish in an oven at 80°C for 2 hours (Figure 13.6).
7. Retrieve the PDMS from the petri dish, cut the sides of the chip and punch hole for the inlets/outlets (Figure 13.7).
8. Place the glass slide and the chip inside a plasma chamber for 2 minutes, the channels in the PDMS chip must be facing upwards (Figure 13.8).
9. Put in contact the PDMS chip and the glass slide. The plasma breaks liaisons at the surface of the glass slide and of the chip. When they are put in contact, new covalent bonds are created and the two parts of the chip are permanently stuck to each other (Figure 13.9).

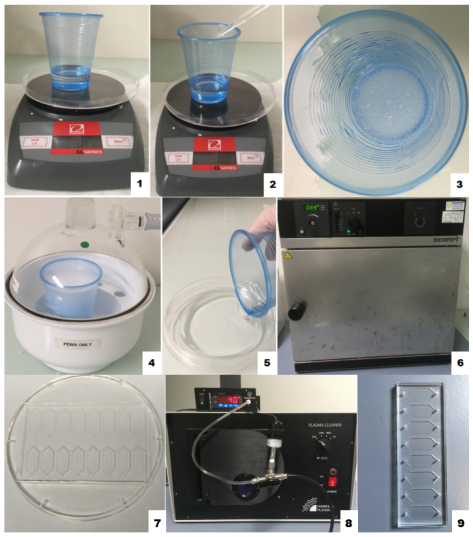

Figure 13: Pictures of the steps of the PDMS chips fabrication process

II.2.2 Molds fabrication using the kit

The techniques detailed in the previous part for the fabrication of the mold are long (at least one hour), expensive and require solvents including a developer which is very harmful.

The idea is to use a single-sided adhesive layer stuck on a glass slide and to use the cutting machine to make the pattern on the adhesive layer (Figure 14). The PDMS can then be deposited on top of the glass side to reproduce the features (Figure 15).

Figure 14: Picture of a mold made of an adhesive film patterned on a glass slide

Figure 15: Picture of a PDMS chip made using a mold made of adhesive

The technical obstacles to overcome were:

- to set the cutting parameters of the machine so that the glass is not carved. Otherwise it would lead to bumps on the sides of the channels in the PDMS chips that can hinder the closing step of the chip.
- the adhesive film should stick well enough to the glass for the mold to be reusable.
- to find a adhesive layer allowing a good enough resolution and that does not prevent the reticulation of PDMS.

For the first point, it is necessary to precisely set the parameters so that the adhesive be cut but not the glass slide underneath. For the vinyl and a pressure of 190 (this is a unitless value, the pressure can be set between 100 and 350 for this machine), the cut was satisfying and the PDMS chips made from the molds were bonded to glass correctly (see Figure 15).
For the second point, this method was first tried with a double-sided adhesive made of polypropylene and polyester liners. This double-sided adhesive had the particularity of being biocompatible, this is not required for a mold but would ensure that the PDMS does not adsorb particles that could end up near the channels afterwards. For a pressure of 300, the cut was satisfying and the resolution was good enough for the chips I had to made. Yet, the top liner would sometimes separate itself from the adhesive when the PDMS chips were separated from the mold (step 7 of the process). Therefore the molds were for single-use only. In order to ensure that the molds be reusable, a single adhesive is used. The bond between the adhesive layer and glass is then sufficient for the mold not to move when the PDMS chip is retrieved.
For the last point, the resolution of the cut is largely dependant on the film's composition: softer and more elastic materials are harder to cut. The blade tends to penetrate the material by deforming it rather than actually cutting it. Vinyl can be cut easily with a

resolution of 200µm. This resolution limits the applications but this remains a good and cheap solution when a relatively high resolution is required.

II.2.3 Proof of concept

Flow wars chips:
To celebrate May the fourth, Elvesys, another microfluidic company with whom we have a lot of interactions, wanted to make a video including a chip which channels spelled "flow wars". This chip was realised using the double-sided adhesive mentioned earlier to make the mold. The mold is shown on Figure 16: the pattern is stuck on top of another adhesive that was stuck inside of the petri dish. This is a possible alternative to using a glass slide that has the advantage of having flexible dimensions. The resulting chip is shown on Figure 17.

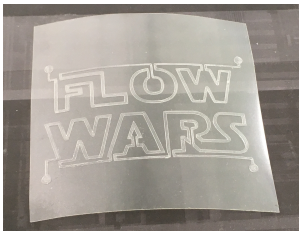

Figure 16: Picture of the mold used to make the "flow wars" chip

Figure 17: Picture of the "flow wars" PDMS chip.

Chambers: A chip was made for a client with a series of chambers. Since the design did not require a small resolution, using this technique was ideal to offer a cheap and fast solution. The mold was made using a 70µm thick vinyl single-sided adhesive with the process detailed in the preceding paragraphs. The mold was realised smoothly (Figure 14) and we were able to stick the PDMS chip to the glass without closing the chambers (Figure 15).

II.2.4 Conclusion

In conclusion, the kit elaborated for paper-based microfluidics can easily be used to make molds with a resolution down to 200µm. A single-sided adhesive should be used to ensure the re-usability of the mold. The established protocol for the mold has been tested successfully.

II.3 Chips made with a double-sided adhesive and a polymeric sheet

The last application of the kit is to create chips using a double-sided adhesive with thin polymeric films on each side (Figure 18). The objective is to develop a process that is fast and requires little equipment.

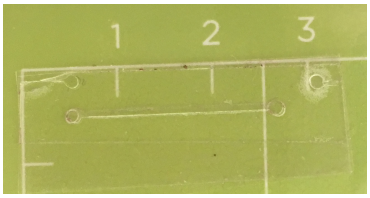

Figure 18: Picture of a chip made of a double-sided adhesive in polypropylene and two films of acetate.

II.3.1 Choice of materials

As mentioned earlier, the resolution and precision of the machine is highly dependant on the material one is trying to cut. Therefore several adhesives with different thicknesses and material composition were bought. For each of them, the design shown of Figure 19 has been used to to determine the resolution and precision according to the direction of the cut. The channels are 1000µm, 500µm, 400µm, 300µm, 250µm and 200µm thick.

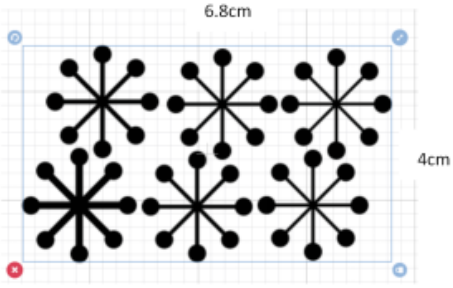

Figure 19: Picture of the design used to determine the resolution and precision of adhesives

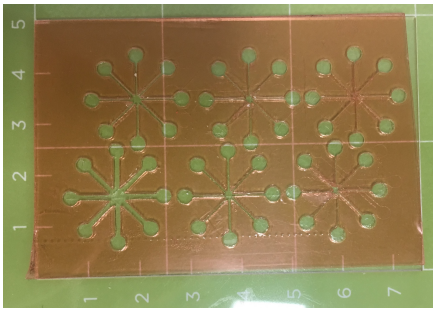

Figure 20: Picture of the 210µm thick adhesive that has been cut according to the design shown on the Figure 19

Four adhesives were tested:

- 90µm thick polypropylene film with rubber adhesive
- 160µm and 210µm thick PET film with acrylic adhesive
- 250µm thick PVC film with acrylic adhesive

The following table summarizes the resolution of the adhesives:

H

Adhesive (thickness)	**Resolution**
90µm	200µm
160µm	300µm
210µm	250µm
250µm	250µm

Table 2: Resolution of the Cricut cutting machine for the different adhesives

This test also revealed that the precision of a cut depends on the direction of the features. Indeed the mechanism of the cutting machine is different for horizontal and vertical displacements. For an horizontal displacement, a rubber chain and a motor make the blade move while for a vertical displacement, a rod makes the mat and the substrate move. The precision was calculated according to the direction and material and spans across a large range which confirms the dependence on the material and direction.

A protocol was established to test whether a given film or adhesive can be cut and if so, to determine the resolution that can be achieved. This protocol can be used by other members of the company in the future to obtain comparable results. It can also be used to test a material for a client which is something offered by the company.

II.3.2 Connectors development

For the chips developed in the previous sections to be functional, we still have to find a way to to connect them to outside equipment at the inputs and outputs in order to introduce and control the flow inside a chip. The idea was to develop connectors that would be repositionable, sustain enough pressure to allow most microfluidic experiments and that would allow two ports of the chip to be close to each other.

The first step has been to evaluate what was on the market, then to come up with a solution and finally to make these connectors and to test them.

State of the art:

- Corsolution [39] sells a system composed of a microscope and connectors that ensure the sealing thanks to a gasket and pressure (Figure 21). This system respects our three criteria but is also very expensive and large.
- Bhagat and Al. [40] offer a solution that is reusable, simple and cheap but that has fixed inlets and outlets positions (Figure 22).
- Atencia and Al. [41] developed a solution with magnets that is repositionable and simple but that would probably close the channels in a chip made of adhesive (Figure 23).

- Several chip holders exist on the market, the chip in placed inside and connected to inlets and outlets (Figure 24). This works very well but does not exist for chips similar to the ones we consider, they are expensive and the ports have fixed positions

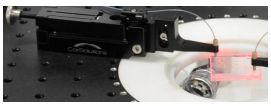

Figure 21: Picture of a connector sold by Corsolution, picture taken from [39]

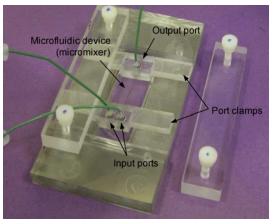

Figure 22: Picture of a connector designed by Bhagat and Al. [40]

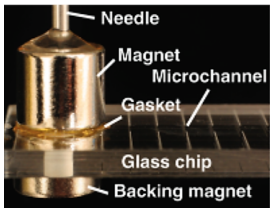

Figure 23: Picture of a connector designed by Atencia and Al. [41]

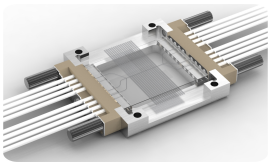

Figure 24: Picture of a connector sold by Dolomite, picture taken from [42]

In conclusion, there exists various systems on the market to connect a microfluidic chip to tubes but none that completely satisfy us. That's why it was necessary to develop our own connectors.

Development of connectors:
To make the connectors, we wanted to find something that is normally closed, that can be removed and placed elsewhere easily, that is thin enough to be able to have ports close to each other, yet larger than tubes. We have opted for metallic tweezers that are 4mm wide at the tip.
The next step was to drill a hole into them to place a small "vacuum cup" inside (Figure 25). This step was initially done manually with a drill but the tweezer is in stainless steel and 1.5mm thick which makes it difficult. The vacuum cup placed in the hole is adapted for tubes commonly used in microfluidics and and is 6mm wide meaning that two ports of a chip have to be separated by at least 6mm. Preliminary pressure tests done at that point have shown that the connector started leaking at 50mbar.
In order to improve the connector we have first placed the chip on a thin plate to make sure that the tweezers remain flat to improve the distribution of the pressure around the port of the chip. The connector was then able to sustain 240mbar on the chip and 900mbar for a PDMS chip which is already a nice improvement and starts being non negligible.
In order to improve the connector further, we have added a small circular gasket (Figure 25) on the vacuum cup. This gasket had two purposes: improve the sealing at the interface with the chip and replace the plate. Thanks to the gasket, we have been able to reach a pressure between 1.5bar and 2bar. This pressure is enough to offer these connectors to clients.
The final connector is shown on Figure 26.

With the final version of the connector, the leak happened between the gasket and the vacuum cup so we have tried fixing that with a stronger glue. Several tests have been done

using the connector and we were able to reach 2bar without any leak. The next step is to find a workshop able to drill the tweezers so that BlackHole Lab can commercialize them.

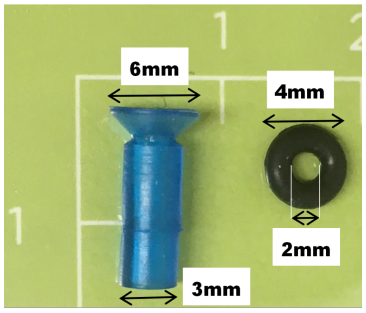

Figure 25: Picture of the gasket (right) and vacuum cup (left) used for the connector

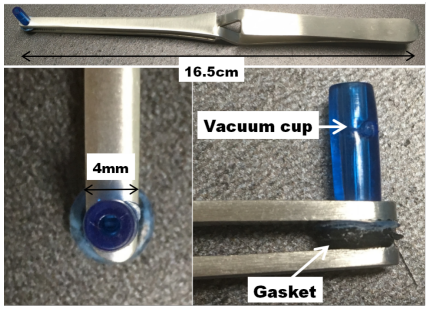

Figure 26: Picture of the connector we have designed

II.3.3 Proof of concept

Now that the connectors have been developed, it is possible to test a chip made of adhesive and polymeric films. The chip we have tested was made of two films of acetate and a polypropylene double-sided adhesive (Figure 18). It was possible to reach 2 bar as mentioned earlier and the leak did not come from the chip.

Figure 27: Picture of a chip made of adhesive and polymeric films in use

II.3.4 Conclusion

Finally, we have been able to develop a process to make microfluidic chips out of a double-sided adhesive and two polymeric films. We have also developed connectors that can be repositioned easily to suit any design, that allow a minimum distance between two ports of 6mm and can sustain a pressure of 2bar.
The limiting factor for this application is the ability of the cutting machine to cut the desired material to the wanted resolution and precision. Indeed, thick film or elastic materials like Flexdym can be difficult to cut with the machine. Another cutting machine, the Cricut Maker may be more appropriate in that case as it comes with blades able to cut thicker materials and can apply more pressure during the cut.

II.4 Other applications

The kit that has been put together to enable the previous three applications can also be used for other applications. Indeed, the cutting plotter can cut other 100 materials according to the brand [43] including most adhesives and thin films as previously exploited. For applications with particular constraints, the kit can be considered to offer an out-of-the-box solution. Here is an example of such situation.

A client needed a basic chip, a chamber, that would be implemented in a preexisting set-up to study a piece of tissue. The piece of tissue is placed on a circular glass cover-slip that has a diameter of 4cm and the chamber should come on top of it. The chamber has to be circular with a diameter of 3.5cm so there is no issue of resolution. Yet, due to the presence of the tissue prior to the bonding between the glass cover-slip and the chip, plasma cannot be used. Therefore a PDMS chip with the process described in the paragraph II.2.1. cannot be implemented.
The solution that was suggested is to use a biocompatible double-sided adhesive in which the chamber is cut out and use a uniform layer of PDMS. The PDMS layer is punched at the inlet and the outlet and is used to make the connections.

To make the chips:

- The cutting plotter can be set so that the top liner and the adhesive layer be cut but not the bottom liner.
- The adhesive inside and outside the chamber is removed.
- A thick and plain PDMS film (about 5mm) is made.
- Circles with a diameter of 4cm are cut in the PDMS layer and holes for ports are punched.
- The top liner of the adhesive film is removed and the PDMS circle is stuck on the adhesive.
- The chip is sent as it is to the client who then has to remove the second liner and stick the chip on to of his glass cover-slip.

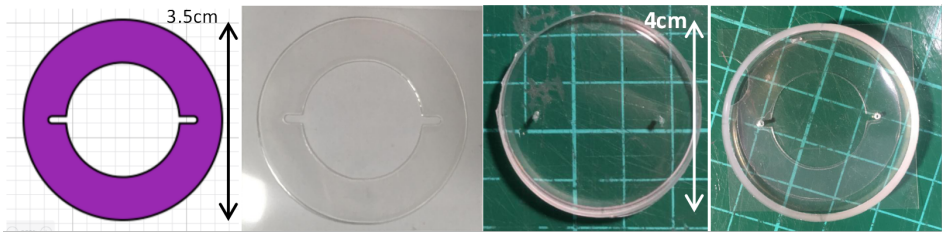

Figure 28: Picture of the main steps of the fabrication process. From left to right: the design used for the adhesive layer; the patterned adhesive layer; the punched PDMS layer; the chip as sent to the client.

Pressure tests have been performed on the chips. For some of them, the adhesive did mot stick well to the glass: a large pressure has to be applied when the top part of the chip is stuck on the cover-slip. A heat treatment can also be done to improve the adhesion: the client can place the chips at 37°C for 15 minutes.
When the adhesive sticks well, 500mbar can be applied in input and the chip was able to withstand 200mbar for two hours without leakage or visible alterations. For a pressure of 1bar, the PDMS is distorted because of the large aspect ratio of the chamber and this induces a shear force that unsticks the adhesive.

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II.5 Conclusion

In conclusion, we have created a kit that is complete and adapted for three applications:

- **Paper-based microfluidic chips**: the kit includes two means of fabrication, xurography and drawing with an hydrophobic ink and the resolution is 300µm which is already small for paper-based microfluidics.
- **Molds for PDMS chips**: using xurography and an adhesive film, molds with a resolution of 200µm can be fabricated. This technique is very simple, cheap and fast.
- **Adhesive-based microfluidic chips**: chips made of double-sided adhesive and polymeric films can be fabricated using xurography. The resolution is also 200µm and the whole process to make a chip is fast, simple and cheap.
  For this application to be possible, connectors have been developed. They are suited for thin chips like adhesive-based ones or regular chips in PDMS and can withstand up to 2 bars which is enough for most applications. The connectors are thin enough so that the ports of a chip can be as close as 6mm apart and the connectors are repositionable and reusable.
- **Others applications**: Lastly, the kit can be used for other applications according to the needs and requirements. This can be achieved by the use of other materials or by making hybrid chips, using an adhesive layer with PDMS or glass for example.

The content of the kit has been though out to ensure that all accessories needed for these previously mentioned processes were included. In addition, some documentation has been written and joined to kit. I have made a datasheet (Annex 1) of the cutting machine and an application note (Annex 2) of the kit that explains how to use the software and how to make the main processes for which the kit was elaborated. Finally, I have written an A4 sheet (Annex 3) summarizing the steps of a process that clients can keep close to the cutting machine.

III. Cells separation by centrifuging forces

Autotransfusion is a process that consists in transfusing to a patient its own blood either during or after a surgery [44]. During that process the cells present in the blood have to be sorted according to their size. The goal of this section if develop a microfluidic chip able to do this sorting.

III.1 Introduction

In this section we are interested in continuous autotransfusion or intraoperative cell salvage [45]: the blood of a patient is drawn, filtered, washed and concentrated before being directly retransfused to the patient during a surgery. This process is used during surgeries like orthopedic and cardiac surgeries where the patient is expected to loose a large amount of blood, at least one liter for adults [46], or when allogenic blood, blood from another person, transfusion is contraindicated (because of religious belief for example) [47].
The main advantage of this technique is that it reduces the need for bank blood which is limited, expensive and in some cases, finding blood matching a patient can be difficult.

The objective in this second part of the internship is to develop a microfluidic chip able to separate cells according to their size. The chip should be able to remove anticoagulent agents previously added during the cell-salvage process on one hand and to remove large particles including small clots on the other hand. Only cells comprised between **4µm and 40µm** should be reintroduced in the patient.
Several cell-separation microfluidic chips will first be described, the chosen technique will then be explained in greater details. The chips fabrication and experimentations will finally be described.

III.2 Cell-separation techniques

There exists many ways to separate cells according to their size using microfluidics. Some are active: they use an external force, a magnetic one for example, while other techniques are passive. In this paragraph, I will describe some of these techniques.

- **Filters**: the simplest way to separate cells is to use a filter. Different kinds of filters can be used to separate cells according to their size: Figure 30 shows three types of filters: weir, pillars or crossflow [48]. The pillars and weirs create an opening large enough for some of the cells to flow but too small for the larger ones. This method is the most basic and simple one, it is also very effective but the filter can get clogged by the larger cells [49].

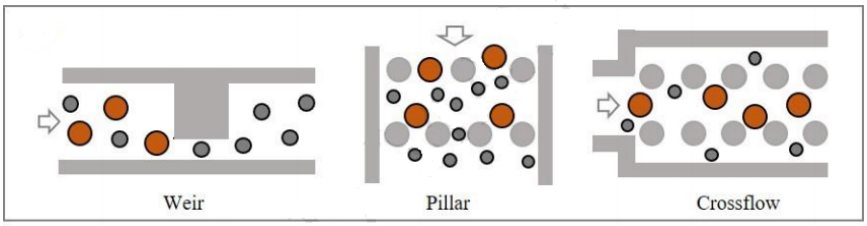

Figure 29: Picture of three types of filters for cell sorting, image taken from [48].

- **Dielectrophoresis**: this active method requires an electric field. In a non-uniform electric field, neutral particles move and their trajectory depend on their dielectric constant as well as their dimension [50]. The **dielectrophoretic force** is given by:

$$F = 2\pi r^3 \epsilon_m \epsilon_0 (\frac{\epsilon_p - \epsilon_m}{\epsilon_p + 2\epsilon_m}) grad|E|^2$$

with r the radius of the particle, ϵ_0 the vacuum permittivity, $\epsilon_m$the absolute dielectric constant of the medium, ϵ_p the one of the particle and E the electric field intensity.

Therefore the dielectric constant determines the direction of the trajectory and the dimensions and dielectric constant determine the amplitude of the displacement.

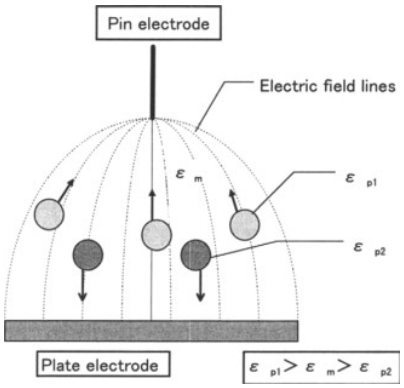

Figure 30: Displacement of particles in a non-uniform electric field according to their dielectric constant, image taken from [50]

- **Deterministic Lateral Displacement**: for this technique, an array of pillars is used to sort cells. The pillars create streamlines shown on Figure 31 (pink lines and colored areas). If the radius of a particle is greater than the streamline width next to a pillar, then the particle escapes the streamline (Figure 32).

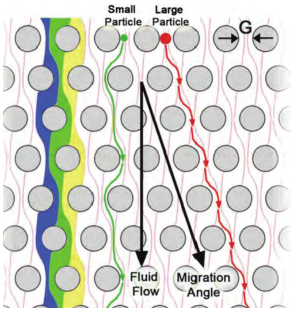

Figure 31: Picture illustrating the streamlines in an array of pillar, picture taken from [51]

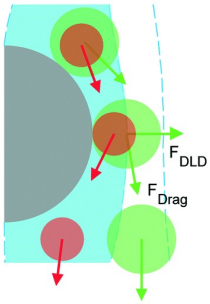

Figure 32: Behavior of particles according to their radius, adapted from [52]

III.3 Spiral inertial microfluidics operating principal

The chosen method uses forces inherent to a curved channel to separate cells.

In order to explain the influence of the different forces involved in a simple manner, cells are considered to be non deformable particles.

In a straight and rectangular channel, the fluid follows a Poiseuille flow with a parabolic velocity profile. The velocity of the fluid is higher in the center of the channel than close to the walls. This causes an **inertial lift force** (F_{IL}) that repels particles from the center. Besides, **a wall induced lift force** (F_{WL}) repels particles from the walls [53][54].

These two opposite forces counter-balance each other and the particles reach an equilibrium position. If $a_p/D_h > 0.07$, with a_p the particle diameter and $D_h = 2hw/(h+w)$ the hydraulic diameter, the equilibrium position is located around $0.2.D_h$ from the walls [54].

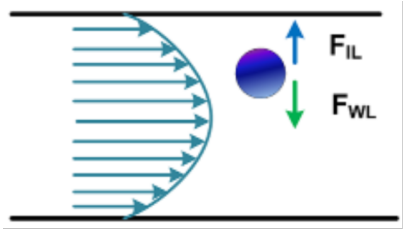

Figure 33: Picture of a Poiseuille flow and illustrating the two forces applied on a particle in a straight channel, image taken from [54]

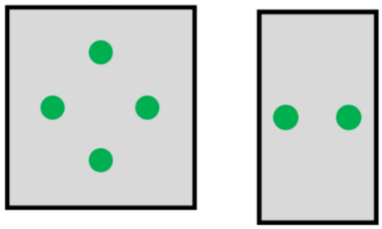

Figure 34: Picture of the focusing positions in a square and a rectangular channel, adapted from [55]

Due to additional lift forces [54], particles don't stay in the corners and the particles' equilibrium positions are are the center of the walls. In the end, there exists four focusing

position for a square channel and two for a rectangular channel (Figure 34).

In a curved channel, a centrifugal force is present in addition to the others and causes the maximum velocity position to be shifted from the center of the channel towards the outer wall of the channel. This induces the apparition of **Dean vortices** (Figure 35, right) and of a **Dean drag force**. The presence of the vortices influences the focusing positions according to the particles size.

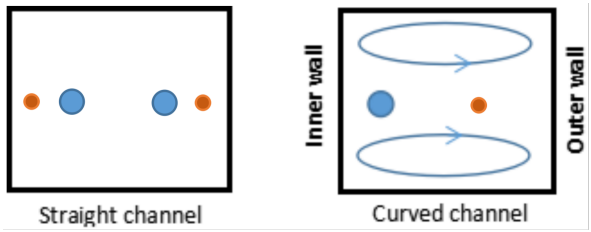

Figure 35: Picture of the equilibrium positions in a straight (left) or curved (right) rectangular channel

Larger particles end up closer to the inner wall of the channel than smaller ones. Therefore the particles are sorted in a spiral channel and it is possible to separate them by creating several outlets to the design. The choice of the dimensions and flow rate enables a calibration of the chip to separate cells or particles of specific size ranges.

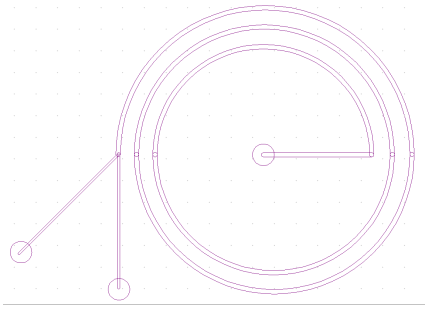

Figure 36: Picture of a design of a spiral channel with two outlets

III.4 Chips design and fabrication

The design visible on Figure 37 was elaborated by Sébastien and the chips were fabricated following the process explained in the paragraph II.2.1.

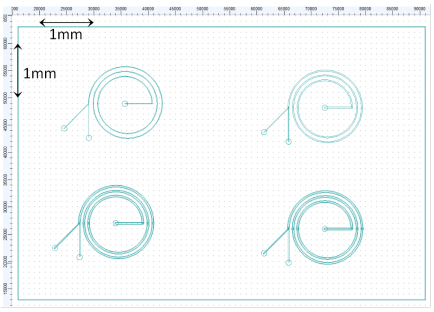

Figure 37: Picture of the designs used to test cell separation

The design is a regular spiral channel with a diameter increasing from 10mm to 13.5mm and the channel width is 100µm, 200µm or 400µm.

III.5 Experimental set-up and preliminary results

III.5.1 Objectives:

The goal of the experiment is to determine the trajectory of particles inside the chip according to their size to be able to adapt the design to sort the particles smaller than 4µm and the ones larger than 40µm. The design shown in Figure 37 has one inlet and two outlet and will be used to determine the trajectory of particles and confirm the theory explained in the paragraph III.3. During the experiment, the impact of the flow rate and of the channel's width will be studied.

III.5.2 Equipment and material:

In order to do the experiment, green fluorescent silica particles with a diameter of 10µm have been used. We had 1mL of these particles diluted in an aqueous solution to a concentration of 50mg/mL. This solution was then diluted fifty times with deionized water to have about one million particles per milliliter [56].

A microscope and a mercury lamp were used to observe the particles and an Ob1 was used to control the pressure at the inlet and the two outlets.

pictures/setup.JPG

Figure 38: Picture of the experimental set-up to test cell separation.

The first experiments have revealed that the silica particles tend to stick to each other as well as to PDMS. This phenomenon has hindered a proper observation of particles trajectory. During this first experiment, some particles have landed in one of the two exit channel along a line. The picture visible on Figure 39 is very promising but this has not been reproduced so it is not possible to tell if this was by chance or resulting from the predicted trajectory.

In order to improve the conditions, a surfactant, TWEEN 20, was added. This has lead to an improvement but particles were still packed together in small groups and some particles stuck to PDMS becoming an obstacle in the trajectory of other particles.

In conclusion these experiments have not lead to an analysis of the trajectory of a particle in the spiral channel yet. Lack of time has prevented me from continuing the experiments further. The dosage of the surfactant should be reevaluated in the next experiments in order to see singles particles flowing in the channel. Once this is achieved, the flow rate should be varied to observe its impact on the trajectory. The comparison of the trajectory in channels with different widths would also help evaluate the impact of the width.

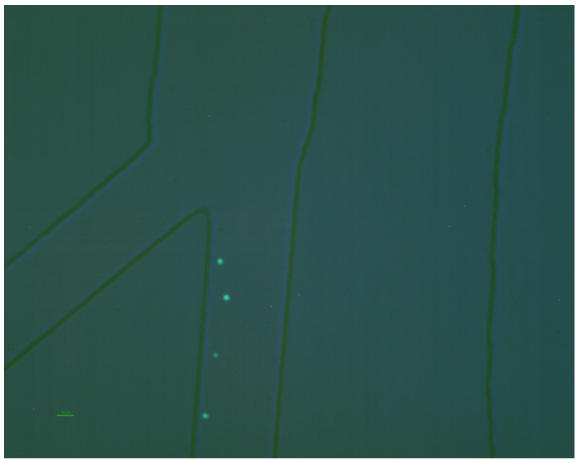

Figure 39: Picture of phosphorous silica particles in a spiral channel. The output channel width is 200µm.

III.6 Conclusion

In conclusion, the experiments started during my internship are to be continued to determine the trajectory of particles within a spiral channel and to determine the design suited to separate cells in three categories: the ones smaller than 4µm, the ones between 4µm and 40 µm and the ones larger than 40µm. Such a chip could then be implemented in a continuous autotransfusion system.

IV. Conclusion

During the internship I have learned a lot about microfluidics, its fabrication methods and possible applications. The first part has consisted in the development of a kit containing all the machines, products and accessories necessary to fabricate microfluidic chips made of paper of using adhesives and polymeric films. The kit can also be used to make the molds necessary for the fabrication of PDMS chips. The elaboration of the kit also included the development of microfluidic connectors that can be used with all chips, included thin and flexible chips such as the ones made of adhesive.

The second part of the internship was focused on using microfluidics to sort cells according to their size. The aim of such a chip is for it to become part of a continuous autotransfusion system. Thanks to a microfluidic chip, the process could start as soon as the first drops of blood are collected whereas there is currently a minimum amount of blood needed to launch the process. I have not had the time to do all the experiments needed and the work is to be continued to properly verify the theory and design the chip.

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Technical datasheet

Cutting machine

Cutting machine

Major strengths :

- Double tool holder to cut and draw in a single step
- Easy to use for personalized microfabrication
- Web software and mobile application
- Bluetooth® technology available
- Small size

A device adapted for microfabrication

- The applied pressure and number of cutting cycles are easily tunable for an optimal cut.
- Can be used to cut a design or locally change the properties of the substrate thanks to the drawing function.

Safety and protection

The device is user-friendly.

Warranty

1 year warranty.

BlackHole·Lab

They trust us

FEATURES

Cutting machine system

This cutting machine is able to cut and draw on light-to-medium weight materials to adapt to your project.

The 30.5 cm by 30.5 cm cutting mat enables you to cut your design on a large sheet or to reproduce it many times at once.

SPECIFICATIONS

Weight	7.26 kg (15.4 lbs)
Dimension (HxWxD)	14x53x14 (in cm)
Power supply	220-240V 50 Hz
Cutting speed	5 cm/s
Resolution	Down to 200µm
Head	- cutting: blades for thin and thick materials - drawing: rechargeable and changeable-ink pen

Phone: 0033(0).782.831.207
E-mail: contact@blackholelab.com
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How to use the xurography machine to design and create microfluidic chips

Cutting machine

Application Note

Sebastian Cargou

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How to use this application note

This application note summarizes the main steps that must be followed for a successful design and fabrication of your microfluidic chips with the xurography machine delivered with your **Xurographykit** from BlackHole Lab.

In order to be as intuitive as possible and straightforward to follow, this application note is organized as follows:

i Type of operation

- Instructions provided here (written **in black**) are necessary and must be executed

Every **blue info box** corresponds to comments and/or tips. It provides useful information and, sometimes, **optional actions**.

Every **red info box** signals a danger. Read attentively before proceeding, and use extra care.

Each step is generally illustrated with an explicit picture.

If you have any question or comment, please contact us!

(See our contact information at the end of the manual)

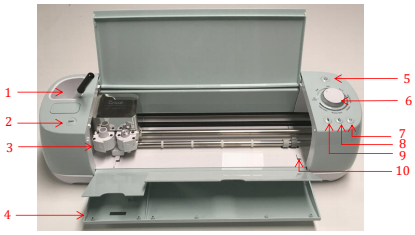
Safety notes:

The aim of this quick-start guide is to illustrate how to use the cutting machine delivered with your Xurographykit and its associated software, so that you can easily and rapidly make your own microfluidic chips. However, it is not intended to replace the manufacturer user manual available online.

The use of the xurography machine is not particularly dangerous for the user. There is no need to have any special equipment or protection.

Table of Contents

I. Machine description



1. ***Pen holder*** to store pens, scissors, etc.

2. ***Open button*** that triggers the opening of the cutting machine.

3. ***Double tool holder*** to hold pens on the left and blades on the right and control their position.

4. ***Two drawers*** to store equipment with special features to maintain blades in place.

5. ***Power button*** to turn the machine on or off.

6. ***Dial*** with 15 positions corresponding to predefined parameters and a «custom» position to modify the predefined parameters or select parameters for a new material.

7. ***Pause button*** to pause the ongoing operation.

8. ***Go button*** to launch the cutting / drawing process.

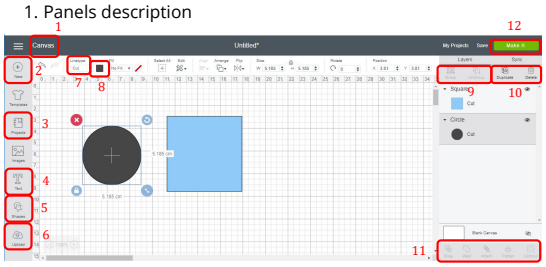
9. ***Load button*** to load the cutting mat prior to the cut / drawing and unload it afterwards.

10. ***Nicks*** to correctly position the mat.

II. Online design software

This software is available online at https://design.cricut.com and requires a free registration to access and save your designs from any computer. It can be used to create or download designs, set the cutting or drawing parameters and launch the fabrication of your chips.

1. Panels description



1. *Canvas:* Opens the canvas page.

2. *New:* Opens a blank canvas to start a new project.

3. *Projects:* Opens preexisting and saved projects. To open a previously saved project, select "**My projects**" in the pull-down menu and "**customize**" if you need to modify it or "**make it**" to print it directly.

4. *Text:* To add text to a design.

5. *Shapes:* To add shapes in the canvas. They can be used to make simple designs which dimensions and orientation can be changed.

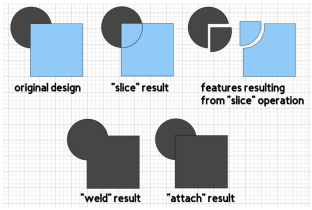
6. *Upload:* To upload designs to the cloud and insert them into the canvas. *See section II.2. for more details.*

7. *Linetype:* To define if the selected design will be cut or drawn.

8. *Color:* To choose the color of the selected design. If two different features have different colors, they will be on two different mats. This means that they are intended to be cut/drawn on two different substrates.

9. *Group/UnGroup:* When several features are selected, they can be grouped. Grouped features can be manipulated at once.

10. *Duplicate/Delete:* Duplicates/Delete a selected feature.



11. ***- Slice:*** Can be used to cut an uploaded design in two: add a feature using "**shape**", superimpose it onto the design and use "**slice**". The design will be cut in two along the edge of the added shape that can be deleted afterwards.

- Weld: Merges two features into one. This is a very important function: for the "original design", the circle and the square will be cut successively whereas only the edge of the "weld result" design will be cut.

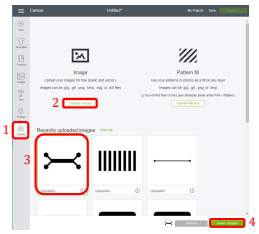
- Attach: Two attached features will be grouped and remain grouped on the mat. It is important to attach the different features of a design if one wants the relative position of the features to remain as such during the cutting/drawing process. If features are not attached they will arbitrarily be placed next to one another on the mat.

12. ***Make it:*** Once the design is ready, click on this button.

2. Importing a design

It is possible to create a design directly in the software using "**Shapes**". This option can be useful to make very simple designs as a first approach or to tune the printing parameters for a new material but the design functions remain limited.

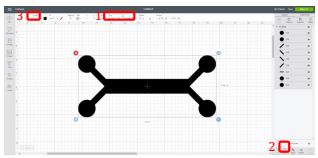
- Once registered, click on "**New project**" then "**Upload**" (1) and "**Upload Image**" (2) to import a design from your computer and "**Save**".



- Select your design in "Recently uploaded images" (3) and click on "**Insert Images**" (4) to add your design to the canvas.

3. Adjusting a design

- Change the size by entering the wanted width or height (in centimeters) in the top menu (1).
- Merge all the portions of the design together so that there are not cut separately by selecting all the portions and clicking on "**Weld**" (2) in the bottom right corner.
- Whether the design should be drawn or cut is defined as the "linetype" (3) on the top menu.



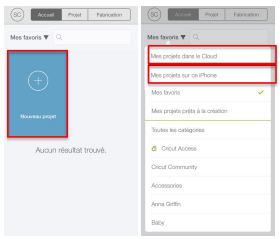
If several designs need to be cut at once, the option «**Attach**» in the bottom right corner can be used to keep the distance between the different parts constant.

- Click on "**Save**" (2) in the top right corner to save your design.

4. Using the software from a smartphone

If the machine cannot be placed next to a computer, a mobile app is available.

a. Home tab

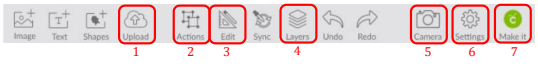


- **New project:** starts a new project, opens a blank canvas

- **My projects on the Cloud / in this phone:** allows you to select a previously saved project from any of your devices. If you select a project, you have the choice to modify or create it. Click on «**modify**» to open the project in the canvas and modify it or «**make it**» to open it in the make tab and print it as it is.

b. Canvas tab

The functions are similar to the ones available in the software but arranged a bit differently.



1. ***Upload:*** To add previously uploaded features to a design, click on "**Upload / Open uploaded images**". The "**filter**" pull-down menu in the top right corner allows you to find the designs available on a phone / in the cloud.

2. ***Actions:*** This is were the functions "**Group/Attach/Weld/Slice/ Duplicate**" are. *See section II.1. for more details.*

3. ***Edit:*** Use this menu to edit a feature. In particular, the size can be defined there, use the padlock to change the height/width separately or not.

4. ***Layers:*** The different layers can be controlled there. In particular, this is where one decides if a feature will be cut or drawn.

5. ***Camera:*** It is possible to use the phone's camera to visualize the mat to help you correctly position the design and the substrate.

6. ***Settings:*** The unit can be changed between centimeters and inches.

7. ***Make it:*** Once the design is ready, click there to place it on the mat then click on "**Continue**" in the bottom right corner. Similarly to the online software, it is possible to set the material and cutting parameters.

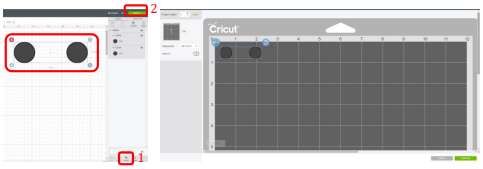
III. Cutting design operation

The machine can either be connected to a computer using the USB cable or a Bluetooth® connection and can be controlled from the online design space. An app is also available to control the machine from a mobile phone with a Bluetooth® connection.

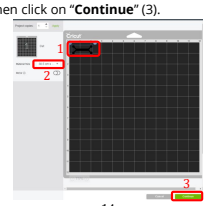
1. Plotting a design

Once your design ready on the design space and saved to the cloud, it is possible to make it using any of your devices.

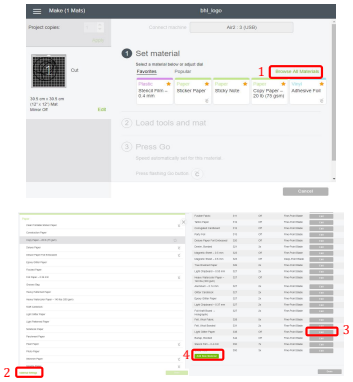
- Upload your design on your canvas if it is not already there.
- Make sure the different portions of a single element are joined together with the "**Weld**" option to ensure that only the outline be cut.
- If your design is made of several and distinct parts, it is necessary to select them and click on "**Attach**" (1) in the bottom right corner so that the spacing between the different parts be respected.



- Click on "**Make it**" (2) in the top right corner.
- Place your design on the mat (1) and select "**30.5 cm x 30.5 cm (12" x 12")**" (2) for the **Material Size** then click on "**Continue**" (3).



- It is possible to use predefined plotting parameters by selecting a material with the **dial** of the machine.
- More predefined settings are available by selecting «**Custom**» with the **dial** and clicking on "**Browse all materials**" (1).
- It is possible to change those settings or to add a new material by clicking on "**Material Settings**" (2) and "**Edit**" (3) or "**Add new Material**" (4).



The xurography machine has to be connected to the computer/mobile phone, using the USB cable or Bluetooth® connection, to be able to define the material and plotting settings.

- Make sure the correct tools (blade and pen) are loaded as indicated on your screen.
- Position the mat using the two nicks on the sides and sliding it in contact with the wheels.
- Press the blinking **Load/Unload button** on your cutting machine.
- Press the blinking **Go button** on your cutting machine.

2. Drawing and cutting

It is possible to use both the functions of drawing and cutting within a single design. For each section of a design, it is possible to assign either "**cut**" or "**draw**" in the "**linetype**" section of the top menu.

- In order for the machine to do both actions at once, the different sections have to be "attached". In that case, after clicking on "**Make it**", the sections will be on a single mat and the machine will first draw then cut your design in a single step.
- It is also possible to do the design in several steps and choose their order. Indeed, there will be as many steps as there are mats and two **unattached** sections with different linetypes or colors will induce two different mats.

The advantage of doing everything in a single step is that, since the sections are "attached", they remain aligned. On the contrary, if one chooses to use different mats, one has to correctly position the different sections on the mats and the alignment won't be as precise but the upside is that it is possible to cut the design first and draw afterwards.

IV. Some examples of use

1. Creating a mask for PDMS chips fabrication

This kit provides a simple and fast way to make your masks for your PDMS process.

- Stick an adhesive film on top of a hard substrate, a glass slide for example.
- Position it on the cutting mat.
- Cut your design in the adhesive layer (left picture).

The cutting settings have to be finely tuned to correctly cut the adhesive layer and leave as little a mark in the substrate as possible.

- Remove the adhesive surrounding the design (middle picture).
- The mask is done !
- PDMS can be poured on top of it to make PDMS chips (right picture).

Design cut in an adhesive layer stuck on a glass slide

Final mold ready-to-use: the surrounding adhesive has been removed

PDMS chip made with the mold

2. Coloring a paper-based design

In order to create supporting features within a design based on cutting paper, it can be interesting to color a feature. Unfortunately, no coloring function preexists on the software and only the outline of a feature can be drawn.

- Create a mask with a set of line as showed below (on the left).
- Create a mask with features to be colored (middle picture).

On the design software:

- Upload the two masks.
- Adjust the dimensions of the main mask to the final ones.

It is not necessary but useful to duplicate the features of the main design one wants to color to ease the manipulations (yellow features on the picture).

- Select all the elements of the main mask and "**weld**" them together.
- Duplicate the second mask as many times as there are features to color, adapt the number of lines to the thickness needed and adapt the dimensions of the lines to those of the features.
- Superimpose the lines on the main mask and change the "linetype" to "**draw**".

BlackHole·Lab

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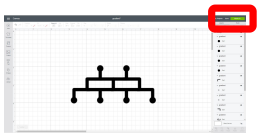
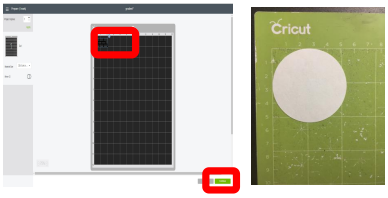
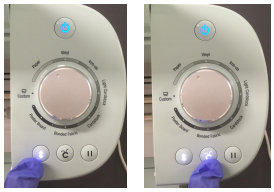
How to / Application notes: Short version

BlackHole·Lab

How to use the Xurography machine?

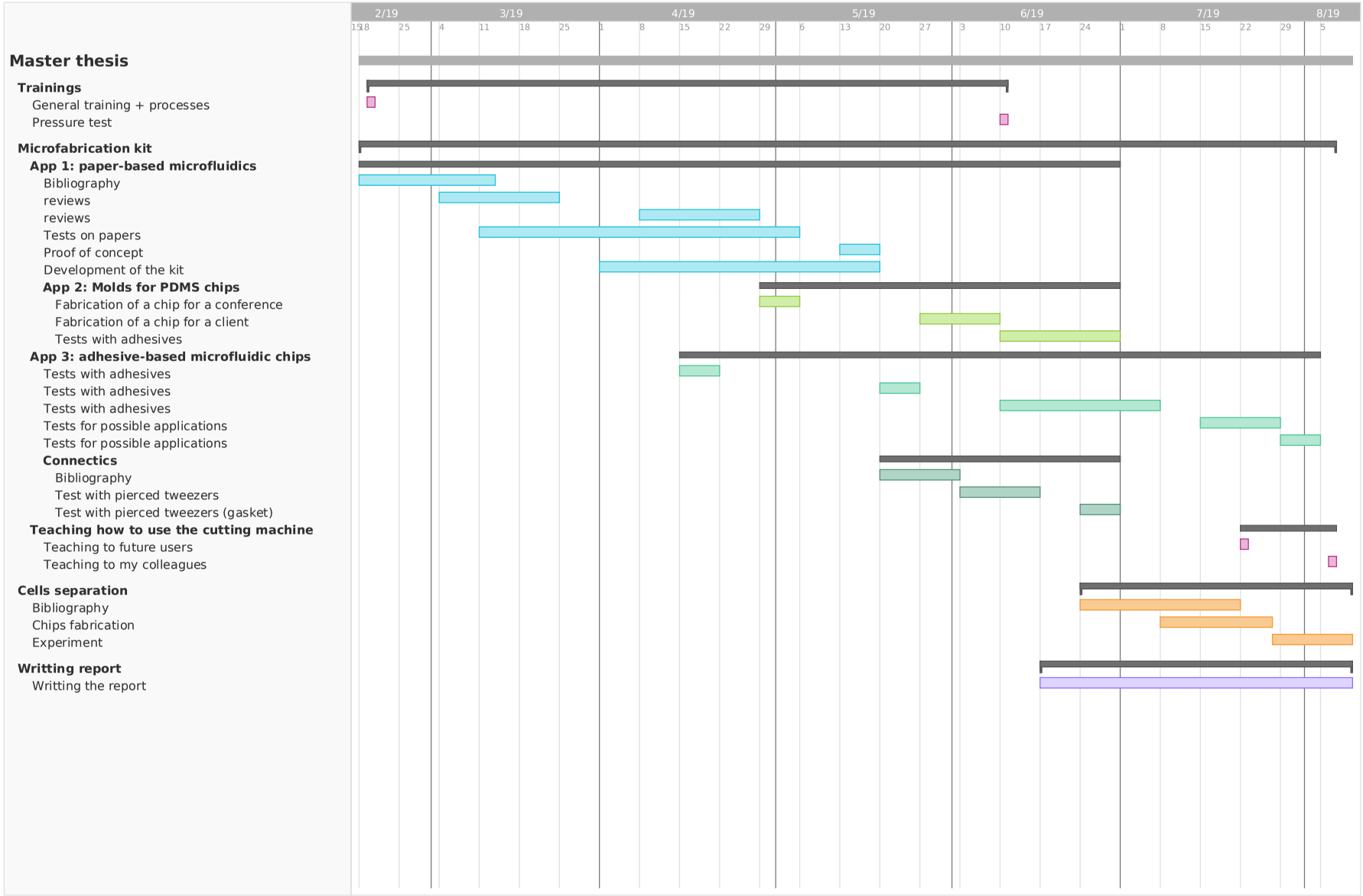
Step	Specification	Visual support
1.	**Switch on** the machine and **open** it. Connect the machine to your computer/phone.	
2.	Set your design and click on **make it**.	
3.	Position the design on the mat on the app and place the substrate on the mat accordingly. Click on **continue**.	
4.	Set the plotting parameters with **the dial**.	
5.	Place the correct blade in the machine (as indicated on the app) and place the mat between the nicks and against the wheels.	 
6.	Press the **load/unload button** and then the **Go button** when they start blinking.	
7.	Press the **load/unload button**.	

For more information, please, read the BlackHole Lab application notes and the manufacturer user manual.

Évaluation du coût du stage

Objet	**Coût [€]**
Salaire	3438.55
Papiers Whatman	30.9
Machine de découpe	318.75
Lames de découpe	46
Tapis de découpe	11.25
Adhésifs	128.33
Colle superglue	5.99
Joints pour pince	1.5
Pinces	30.25
Fraises pour perçage	40.65
Encre hydrophobe	24.78
Marqueurs	14.36
PDMS	120
Emporte-pièces	45
Eau distillée	2.3
«Vacuum pumps»	30
Lames de verre	20
Guide-doigts	4.99
Particules fluorescentes	17
Tween 20	10
Consommables divers	50
Total	**4326.6**

ANNEX 4: Gantt diagram



BlackHole·Lab

Master thesis

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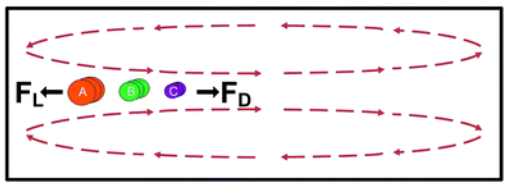
THE COMPANY: BlackHole Lab is a startup in microfluidics founded in 2014. It is part of a consortium of French companies working on microfluidics called "La vallée microfluidique". BlackHole Lab is an equipment distributor for laboratories and companies to enable them to make microfluidic chips on their own. The company sells kits conceived to contain everything necessary to do a process and sold with a training, documentation written to explain the processes step by step and assistance if additional help is needed. All the processes are designed to be performed outside a clean room.

STUDY OF A MICROFLUIDIC CHIP FOR AN AUTOTRANSFUSION PROCESS

Continuous autotransfusion or intraoperative cell salvage: the blood of a patient is drawn, filtered, washed and concentrated before being directly retransfused to the patient during a surgery.



During the intraoperative cell salvage process, cells larger than 40µm (clots...) and cells smaller than 4µm (anticoagulent agents...) have to be taken out of the blood.

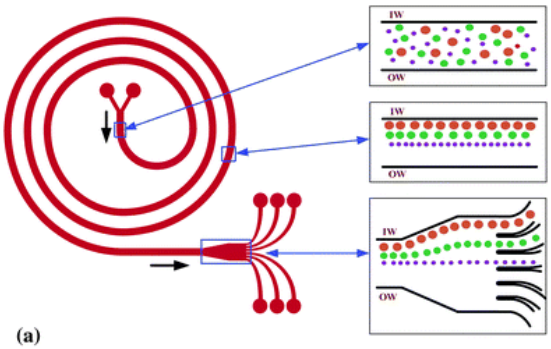


Image taken from [1]

CELLS SORTING

In a curved channel, lift forces and Dean drag force are opposed and lead to an equilibrium position that depends on the size of the particles.

[1] : Kuntaegowdanahalli, S. S., Bhagat, A. A. S., Kumar, G., & Papautsky, I. (2009). Inertial microfluidics for continuous particle separation in spiral microchannels. Lab on a Chip, 9(20), 2973

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Master thesis

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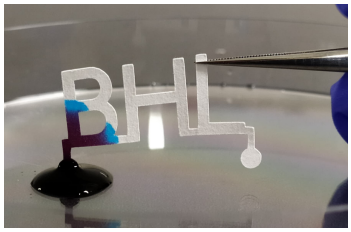
DEVELOPMENT OF FABRICATION PROCESSES FOR PAPER-BASED MICROFLUIDICS AND ALTERNATIVE POLYMERS TO PDMS

During the internship, I have developed a kit based on **xurography**: the use of a cutting plotter to fabricate microfluidic chips. The kit contains the equipment and accessories needed for three possible applications:

Paper-based microfluidic chips:

Main advantages: cheap and simple, portable and robust, easy to use, disposable, biocompatible

Resolution: 300µm



Molds for PDMS chips:

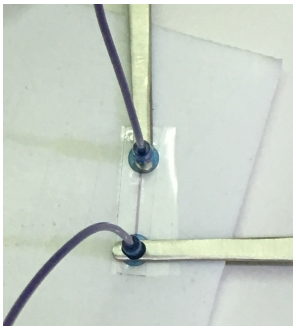
A single-sided adhesive film is stuck on a substrate and patterned using xurography. PDMS can then be poored on top of the mold following the conventional process.

Resolution: 200µm

Adhesive-based microfluidic chips:

Using xurography, chips can be fabricated using a double-sided adhesive and polymeric films on each side.

Resolution: 200µm



Abstract

Xurography, the use of a cutting plotter in microfluidics, is one of the many techniques of microfabrication that can be used to make paper-based microfluidics. This report describes the development of a commercial kit suited for the fabrication of paper-based microfluidics chips, the creation of molds for PDMS chips and the fabrication of microfluidic chips based of adhesives. Connectors able to sustain 2bar are developed as well. These connectors are reusable, repositionable, allow two inlets or outlets to be as close as 6mm apart and are suited for all kinds of chips, included the ones based on adhesives. The achievable resolution of the kit is 200µm for polymers and 300µm for paper.
The second part of the report focuses on the use of microfluidics to sort particles according to their size in order to design a chip that could be introduced in a cell salvage system: a system used for continuous autotransfusion. The microfluidic chip in question uses a spiral channel for the separation. The state of the art and some preliminary experiments are detailed in this report.

Résumé

La xurographie, utilisation d'une machine de découpe pour fabriquer des puces microfluidiques, est l'une des techniques de microfabrication possibles pour faire des puces microfluidiques en papier. Ce raport décrit la création d'un kit commercial dédié à la fabrication de puces microfluidiques en papier, de moules pour la fabrication de puces en PDMS ainsi qu'à la fabrication de puces réalisées à l'aide d'adhésif et de films en polymère. Des connecteurs microfluidiques capables de résister à une pression de 2 bar sont également conçus. Ces connecteurs sont réutilisables, repositionnables, permettent à deux entrées ou sorties d'être séparées de 6mm au minimum et sont adaptés à tout type de puces microfluidiques, y compris des puces très fines. La résolution de ce kit est de 200µm pour des adhésifs et films en polymères et de 300µm pour le papier.
La seconde partie du rapport concerne l'utilisation de la microfluidique pour trier des particules en fonction de leur taille et l'introduction d'une telle puce dans un système d'autotransfusion continue. La puce microfluidique en question utilise un canal en forme de spirale pour la séparation des cellules. Un état de l'art ainsi que des expériences préliminaires sont détaillées.

Abstract

La Xurografia, l'utilizzo di un plotter di taglio in microfluidica, è una delle numerose tecniche di microfabbricazione che possono essere utilizzate per realizzare microfluidi su carta. La presente relazione descrive lo sviluppo di un kit commerciale adatto alla fabbricazione di microfluidi su carta, la creazione di stampi per chip PDMS e la fabbricazione di chip microfluidici a base di adesivi. Sono sviluppati anche connettori in grado di sostenere 2bar. Questi connettori sono riutilizzabili, riposizionabili, permettono due prese o prese di essere vicine a 6 mm di distanza e sono adatti per tutti i tipi di chip, inclusi quelli a base di adesivi. La risoluzione raggiungibile del kit è di 200µm per polimeri e 300µm per carta.
La seconda parte della relazione si concentra sull'uso di microfluidi per selezionare le particelle in base alle loro dimensioni al fine di progettare un chip che potrebbe essere introdotto in un sistema di recupero cellulare: un sistema utilizzato per l'autotrasfusione continua. Il chip microfluidico in questione utilizza un canale a spirale per la separazione. Lo stato dell'arte e alcuni esperimenti preliminari sono dettagliati in questa relazione.