Paper-based microfluidic sensing devices fabricated by inkjet printing technology

Bernat Guirao Expósito
Master's Thesis Advisor: Daniel Citterio
Analytical Chemistry laboratory
Department of Applied Chemistry
Keio University

Chemical Engineering Master's Thesis
Validated by ETSEIB (UPC)

Index

1. Abstract

The detection of some analytes, for example heavy metal ions, is crucial in locations such as developing countries or humanitarian emergencies, where no qualified professionals or sophisticated equipment is available.

In this project, a detection system using inkjet printing technology is developed. The system relies on printing of hydrophobic UV-curable inks to define microfluidic channels in paper, and to print a sensor inside this channel. After that, a solution sample can be dropped into the channel, resulting in a colour change of the sensor. By digitally measuring the colour change, the analyte quantity can be determined. Actually, the main focus of the project is the testing of different channel geometries in order to improve their performance.

The aim of this project is not only to develop a detection system, since nowadays a lot of them are already available. The system proposed here requires neither qualified professionals nor very sophisticated equipment for operation. Actually, the goal is to detect with as simple equipment as possible. Only a computer, a commercially available printer and a colour scanner would be sufficient, making it possible to use them in areas such as developing countries or in humanitarian emergencies. Besides, this detection system has been designed to be as environmentally friendly as possible.

2. Introduction

The system studied in this project, which is explained in *3. Experimental section* in more detail, consists basically in printing a hydrophobic microfluidic channel in a piece of paper, creating a barrier that does not let hydrophilic liquids to escape from the channel created. Then, the sensor reagent is printed inside the channel, immobilizing the sensor reagent and requiring small quantities of it. Once the system has been created, analyte is dropped into the channel. When analyte sample reaches the sensor reagent, colour change is detected. It can be measured with a scanner and a simple colour detection program. From the colour obtained, analyte parameters such as concentration can be deducted.

The system proposed is simple and low price. It only requires using a printer to print the reagent sensor and an availably commercial scanner to scan the paper and detect the colour change. Actually, a person with only basic computer skills is able to use this system. In developing countries there is a lack of qualified professionals, so it is crucial to design an easy to use system.

Otherwise, industrial fabrication of the system is cheap, since inkjet printing technology and UV light are available technologies in the industry. Moreover, the system is made of paper, which is an available and cheap material, and the other reagents, basically the ink and sensor ones, are expensive, but only small quantities are used.

This system presents an incredible amount of applications. Actually, there are as much applications as sensor reagents that are able to be printed into the channels. Besides, the system is cheap and easy to use, so no qualified professionals or large amounts of money are required to use it, which increases the applications. Some examples of applications are on site detection or analyte properties quantification in humanitarian emergencies or in difficult access zones (applications in exploration and military fields) as well as household applications.

It must be taken into account that more research must be conducted in this project field. So, in the nearly future it may substitute some current applications of well established and commonly used technologies such as photolithography.

2.1. Paper patterning technologies

Printing technologies have experienced a great development during the last decades, and they are no more exclusive to the publishing sector, since applications in fields like chemistry and biology, for example, have been found. [19]

Actually, paper-based sensor fabrication is one of the research fields where printing technologies are being applied. The idea of making sensors using paper-based technologies is very attractive, since they are easy to use and may provide valuable information. However, a lot of research is needed to make them cheaper and more precise, since they often require so expensive technologies and the information provided is almost qualitative and not as exact as desired.

Most of paper-based sensors do not require external equipment or reagents. So, this kind of sensing devices are adequately inexpensive to be commonly used, including in developing countries, in emergency first aid situations, and in home health-care settings, in contrast to conventional medical diagnostic technologies that were designed for air conditioned laboratories, highly trained personnel, a constant supply of reagents, and considerable volumes of sample. Besides, low sample and sensor volumes are required, which is important in the case of available clinic samples or high cost sensing reagents.

Among the paper patterning technologies available, inkjet printing, photolithography or wax printing, among others, may be useful for sensor fabrication. In this project inkjet printing technology was selected as the most appropriate one. However, important advances are taking place on the other technologies, too.

2.1.1. Photolithography

In the photolithography field, a lot of research has been conducted, trying to apply it to paper-based sensors.[1] Photolithography consists in using a light to transfer a geometric pattern from a photo mask to a light-sensitive chemical. One of the main problems it presents are its high cost, since sophisticated equipment and qualified professionals are needed, making it more expensive to implant in mass scale production than other printing methods, and being almost impossible to be applied in developing countries. Besides, the substrate used must be chemically treated, including the hydrophilic part, implying a substrate contamination risk. However, important improvements and research are being conducted to solve these problems, trying to make it a more feasible method.[2]

2.1.2. Wax printing

Another printing technology used regularly is wax printing. Actually, wax printing is a promising technology in the fabrication of microfluidic paper-based analytical devices, since printing velocity is extremely high and it is feasible to use it for mass scale production. For example, wax printing takes less than 3 min to prepare four multizone plates, while photolithographic methods require about 20 min to prepare a single plate.[3] Moreover, wax printing is a flexible technology, so it is possible to change the fabrication process easily, like the design of the patterns.

The system consists basically in printing wax on the paper and heat it in a hot plate, penetrating the molten wax into the paper, creating a hydrophobic barrier. It must be taken into account molten wax penetration into substrate makes the channels wider than in the original design, although some models exist to predict channel widening.[4] Otherwise, the same phenomenon occurs in inkjet printing technology.

In terms of producing industrially paper based microfluidics, wax printing could be a feasible technology, due to its velocity, as it was told before, and simplicity and nature of the process itself. However, another technology should be used to print the sensor reagent, like inkjet printing technology. And creating a hydrophobic barrier could be complicated due to wax solvent compatibility problems.

2.1.3. Inkjet printing technologies

Inkjet printing has become a useful and feasible technology in a lot of industrial fields for accurately depositing very small quantities (tens of picoliters) of materials at defined spots on the surface of a wide variety of substrates. Moreover, it is a single step process which does not require highly qualified professionals or an important amount of facilities, in contrast to photolithography. And it is a suitable technology for scale industry mass production, too. So, its time saving, low cost and flexibility will probably establish inkjet printing technology as one of the most suitable printing technologies in the future. Furthermore, modern inkjet printing devices have multiple print heads which are able to print different kinds of ink simultaneously, being able to print both the hydrophobic barrier of the pattern and the sensor reagent, and, in contrast with other printing technologies like plotting, the place, time and quantity of liquid deposition can be controlled precisely ("on demand" inkjet printing technology).

In spite of all the advantages mentioned, inkjet printing technology has been mostly applied in plastic electronics and for the manufacture of polymer light-emitting diodes[5-8], and in the analytical chemistry field only in the fabrication of electrochemical sensing devices.[9-10]

Inkjet printing technology is also a feasible technology for the fabrication of microfluidic sensing devices incorporating various chemical functions.[11-12]

2.1.4. Other paper patterning technologies

Although other technologies for patterning paper exist, they are not as important as the previous ones. Some examples may be cutting and plotting.

In the case of cutting, the knife cost is representative and sample and sensor are not isolated in a zone of the paper, getting wet all the paper when making analysis, and consequently being able to contaminate people and objects around.[13]

Regarding plotting technology, it is not readily adoptable to the dispensing of the reagents required for chemical sensing. Moreover, plotting technique is a continuous line drawing method, with the amount of dispensed liquid being more difficult to control and, therefore, limiting the resolution of plotted structures to 1 mm, approximately. Besides, lately felt tip-based x,y-plotters are being replaced by inkjet printing devices, being gradually more difficult to find in the market [14-15].

2.1.5. Paper patterning technology election

Definitely, in order to achieve properly this project goals, inkjet printing is the most suitable technology, since it is simple, cheap and flexible and it can be used in emergency and first aid situations. Moreover, it is feasible to apply it in mass production scale and multiple print heads can be used to print both the hydrophobic barrier of the pattern and the sensor reagent.

2.2. Substrate: paper

Different substrates can be used in microfluidic sensing devices, such as glass or paper. In the case of this kind of sensing devices, the substrate must let the analyte to flow through it, so it would have a porous structure or some external equipment, such as external pumps, would be used. Paper has a porous structure, since it is normally made of cellulose fibers. But paper also has more advantages, since it is abundant, inexpensive (6$/m^2 even for high-quality chromatography paper), sustainable, easily and environmentally friendly disposable, easy to use, store, and transport and easy to modify chemically.

Paper is also a familiar material and everybody is used to it. This is an important factor, since one of the final goals of this research branch is making sensing devices to be used in developing countries or emergency situations, so no qualified professionals must be able to use them easily. But perhaps its most important advantage is that an enormous variety of inkjet printing techniques, concretely inkjet printing technology, is available for its functionalization. Moreover, paper has been used in the analytical chemical field for so many years, and a lot of experience working with this material has been acquired, so a lot of advantage can be taken from existing analytical techniques.

Paper physical properties make it very useful for chemical analysis, too. It is thin, lightweight (10 mg/cm^2) and available in a wide range of thicknesses (0.07-1 mm). Moreover, it is easy to stack, store, and transport. Its composition and structure makes it feasible to work with biological samples, since it is made of cellulose or cellulose-polymer blends, and it can be modified chemically to incorporate a wide variety of functional groups that can be covalently bound to proteins, DNA, or small molecules. Another advantage is the fibrous nature of paper, which provides to the system intrinsic capillary pumping[18], whence no external pumps or similar sophisticated equipment is not required. Moreover, nowadays printing techniques are able to make sophisticated microdevices which incorporate more functionality, for example metal electrodes, without the need of clean rooms or other kinds of expensive infrastructure.

Paper colour is white (it scatters light), so it provides contrast with a coloured substrate, being an available substrate for colorimetric tests, and it is available in a wide range of highly engineered forms with a very wide range of properties. For example, filter papers with well-defined pore sizes can separate suspended solids from samples before an assay or remove erythrocytes from blood.

From an industrial point of view, paper can be easily manufactured on a large scale by the well-established coating and printing techniques[17], which can further lower the cost of the final products. Moreover, little initial investment would be required.

2.3. Quantitative detection of analytes

Quantitative colorimetric detection of analytes is possible by reflectance detection when the intensity of the colour that develops in the test zones is a function of the concentration, or another parameter, of the analyte. In other words, the colour change produced in the sensor due to the contact with the analyte can be detected, and with this information and a calibration curve, the analyte desired parameter can be calculated. As it was told before, the system designed must be easy to use, so that no qualified people can use it. To see the colour change with the naked eye may be impossible in some cases, so simple systems like scanners and colour registering software may be used.

2.4. Previous research

Whitesides and coworkers introduced the concept of using patterned paper substrate as a microfluidic platform for multiplex analyte detection. In their studies, hydrophobic polymers were photolithographically patterned[19] or printed using a desktop plotter[20] onto hydrophilic paper so that the millimeter-sized fluidic channels can be well-defined in the paper matrix. Owing to the natural capillary action of paper, fluids can flow along these channels without the external pumps that are required in the conventional microfluidic devices. The use of these patterned paper platforms minimizes the use of sample volume and makes multiple analysis possible. Whitesides and coworkers were able to detect glucose and protein simultaneously on a single patterned paper. In a more recent work[21], an imaging device (camera phone or portable scanner) was added to the system in order to make quantitative analysis possible.

Actually, inkjet printing can be used to deposit a wide variety of materials, such as inorganic particles or biological species, on paper. For example, Pelton *et al*. coated biomolecule-attached microgel inks on paper[22] and Abe *et al*. printed antibodies to analyze biological parameters[11].

The concept of the system used in this project is similar to the system used by Abe *et al.*[12]. That system consisted in soaking filter paper in a 1.0% wt solution of polystyrene in toluene for 2h and then drying at room temperature for 15 minutes. This turned the paper, which is hydrophilic, in hydrophobic. Then, a hydrophilic pattern was etched by the ejection of toluene droplets, which dissolved the hydrophobic polymer material. The dissolved excess polymer was accumulated at the outside of the liquid droplets according to the coffee ring effect upon toluene evaporation and taken up by the surrounding paper. Doing this, the paper became hydrophilic again, almost exactly as at the beginning. Once the hydrophilic channel had been created, sensor reagents could be printed using inkjet printing technology, too. Using this method, multiple analyte sensors can be done. However, this system has been criticized due to the risk that some hydrophobic polymer remained in the hydrophilic pattern, since at the beginning all the paper was soaked.

In figure 1, a schematic representation of the system used by Abe *et al.* to make multianalyte sensors is shown.

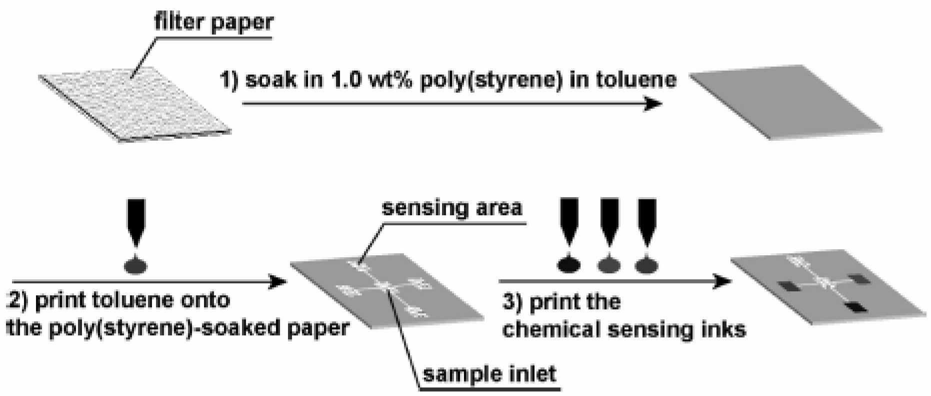


Figure 1. Schematic representation of the system used by Abe et al. to make multianalyte sensors. Source: Abe et al., 2008 [12]

The main problem of this system was the coffee ring effect that took place when the analyte reached the sensor. The analyte pushed the sensing reagents to the sensing area limits, resulting in a non homogeneous colour, which made difficult to take out valuable information of the experiments. In figure 2, an outline describing the coffee ring effect consequences can be observed.

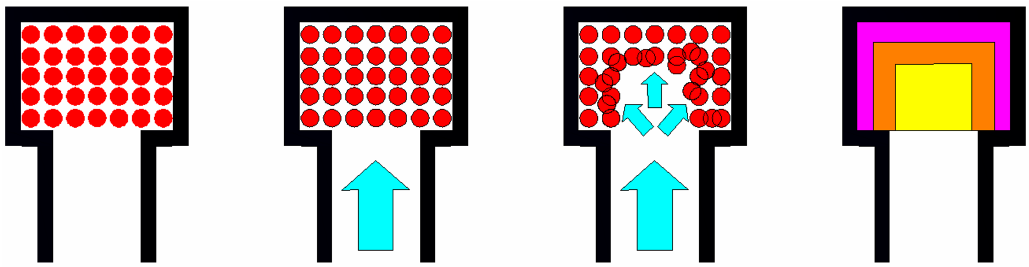


Figure 2. Outline showing the coffee ring effect consequences

In figure 3, the sensor reagent can be observed before and after application of the analyte. The effects of coffee ring effect can be observed, too.

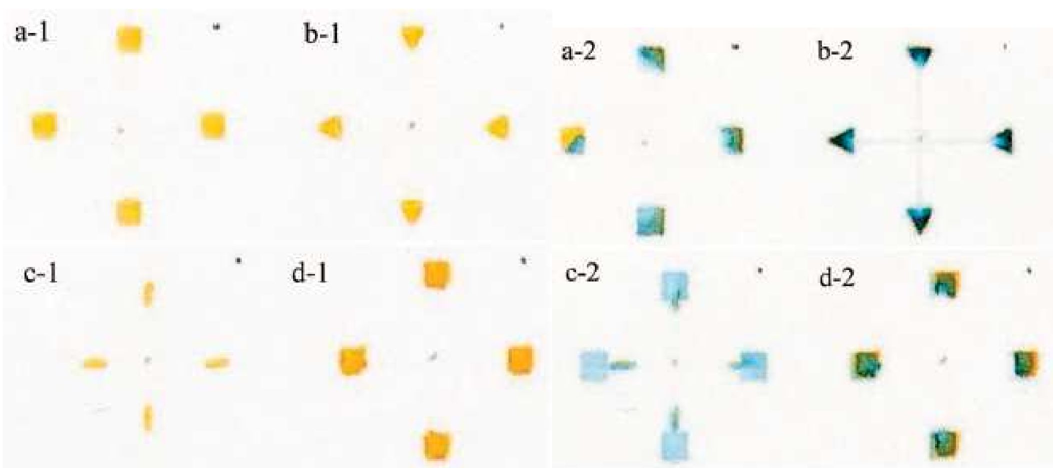


Figure 3. Sensor reagent in different sensing areas, before (patterns numbered with "1") and after (patterns numbered with "2") analyte application. Source: Abe et al., 2008 [12]

3. Experimental section

3.1. Preparation of the patterns

The system proposed to make patterns proposed by Abe *et al*.[12] used toluene, which is an environment and health affecting volatile organic solvent (VOC) that requires some security measures when it is used. Moreover, it had the problem that the analyte flowed for a path where previously a polymer had been printed. Despite polystyrene was removed using toluene, which vaporizes easily, some contamination may occur, leading to incorrect results. So, a new method of creating patterns was used, which consisted basically in printing the hydrophobic barrier only in the parts required to be hydrophobic. Doing this, the hydrophilic part where the analyte had to flow remained unchanged, no risk of contamination existed, and toluene was not used.

The reagents used to create the hydrophobic barrier were changed, too. One of the reagents used previously was toluene, but low vaporization temperature solvents are not desired reagents in the industry field, since they are considered dangerous. So, instead of the toluene and polystyrene solution, a solution of Octadecyl Acrylate, 1,10-Bis(acryloyloxy)decane and 2,2-Dimethoxy-2-phenylacetophenone, which was used as initiator, were used.

Once the solution was printed, it was polymerized using ultraviolet light (Hamamatsu Lightning Cure 6686). In all the experiments 600 mW/cm^2 were used. It must be taken into account that once this solution was printed, it spread very fast, so UV light must be applied immediately after printing. If some time was awaited between printing and polymerization, spread of the ink would be produced, and when it was polymerized, the pattern would get a different form from the original, causing symmetry and flow problems.

It must be taken into account that the hydrophobic barrier printing procedure consisted in two steps, since the front part and the back part of the substrate were polymerized independently. The back part of the pattern was totally covered by the hydrophobic barrier, to prevent the sensor reagent and the analyte from leaking outside the pattern.

Once the hydrophobic pattern had been printed, the sensor reagent could be printed in the desired zone of the pattern. Once both the hydrophobic barrier and the sensor reagent were printed, analyte was dropped into the pattern and some time later colour change was analysed using a colour detection software (Digital Color Meter software Ver. 3.6.1 provided with the Mac OS X Ver. 10.5 operating system).

The substrate used to create the patterns was filter paper (Advantec 5C 185mm). The substrate used must be absorbent and resistant to UV light.

In Figure 4, a schematic representation of the preparation of the patterns is shown.

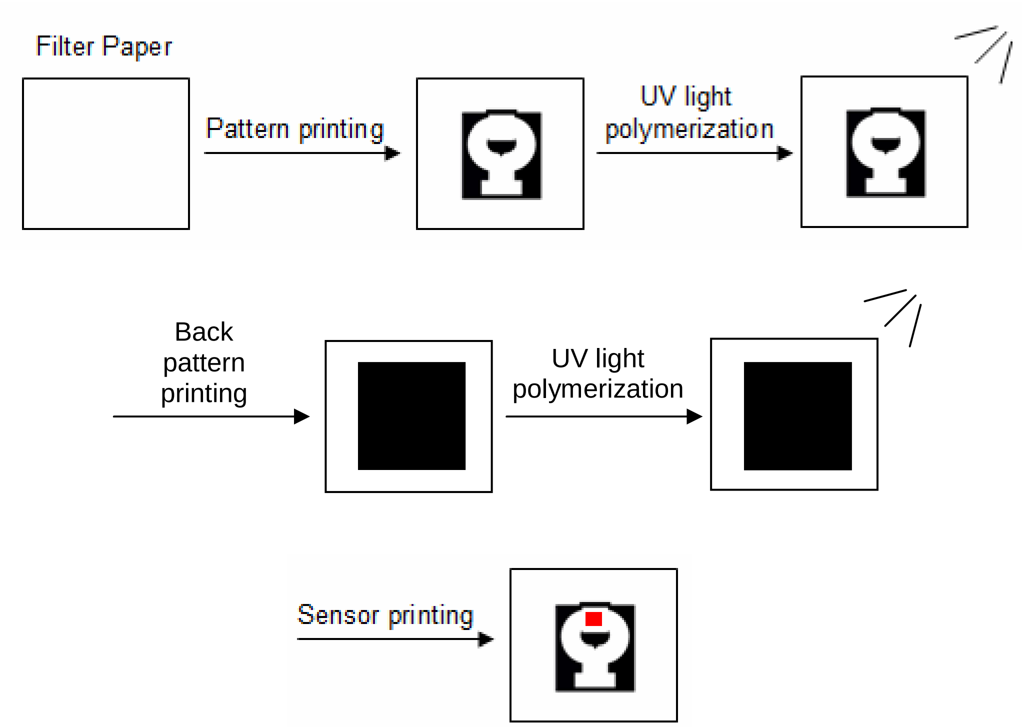


Figure 4. Representation of the preparation of the patterns

3.2. Preparation of the hydrophobic ink

As it was told before, the solution used to create the hydrophobic barrier consisted of Octadecyl Acrylate (ODA, provided by WAKO), 1,10-Bis(acryloyloxy)decane (DDA, provided by Tokyo Chemical Industries) and 2,2-Dimethoxy-2-phenylacetophenone (provided by WAKO), which was used as initiator. To prepare it, a solution of ODA and DDA (ODA-DDA, 70-30 %wt) was prepared, and later initiator was added to the solution (ODA:DDA-initiator, 85-15 %wt). Then, some stirring time was applied.

This solution showed good performances at short time term, but after some days the pattern performances clearly worsened. In order to improve the pattern durability, the stabilizer of ODA and DDA can be removed.

To remove the ODA stabilizer, it was mixed in an ethanol and 5% wt NaOH solution, and it was submitted to a separation process. After the separation process, distiller and a vacuum bomb were used to evaporate the remaining ethanol. In the case of DDA, the process was the same, but no ethanol was used.

3.3. Preparation of the pH sensor

Two pH sensors were used in this project, one made of particles and another one without them. In the first experiments made with pH sensors, the particle based sensing reagent was used, since the capacity of particles to get stuck to the paper made it more resistant to the coffee ring effect. However, it has been demonstrated yet that coffee ring effect can be avoided using pH based particle sensing reagent even with the simplest patterns (the ones with only an analyte entrance, such as the “Simple test” series patterns). Preparation method of this reagent is explained by Abe *et al.*[11]

Non particle pH sensing reagent, which is more easily affected by the coffee ring effect than the previous one, was used to demonstrate that multiple path channels can avoid it. Preparation method of this sensing reagent is explained by Abe *et al.*[12]

3.4. Preparation of the pH buffers

pH buffer solutions from 5 to 9 were created in order to test the patterns and create a pH calibration curve with the results. Their respective compositions are shown at table 1.

Buffer solution	Reagents used		
	Distilled water	**Citric acid ($C_6H_8O_7$)**	**$Na_2HPO_4 \cdot 12H_2O$**
pH 5	40 ml	0.3765 g	1.4612 g
pH 6	40 ml	0.2881 g	1.7907 g
pH 8	40 ml	0.0215 g	2.7848 g
	Distilled water	**Na_2CO_3**	**$NaHCO_3$**
pH 9	40 ml	0.0424 g	0.3024 g

Table 1. Composition of the pH buffer solution used

To create the pH 7 buffer solution, pH 6 and pH 8 buffer solutions were mixed.

3.5. Geometry of the patterns

As it was commented on the Previous Research section, the main problem of printing patterns on the sensing areas was the coffee ring effect. In order to avoid it, patterns with two, or even four, channels entering the sensing area were investigated. The basic idea was to make symmetric flows to enter the sensing area and cancel among them when interacting with each other, so that the sensing reagent would become wet, but it would not spread through the channel, offering an homogeneous colour.

In figure 5, a pattern with two symmetric paths arriving at the sensing area are shown.

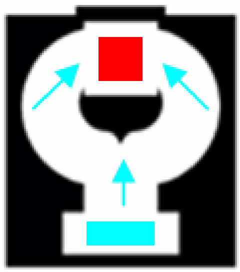


Figure 5. Pattern with two symmetric paths that arrives at the sensing area. The blue zone is the dropping analyte zone and the red one is the sensor reagent one.

The pattern geometries were designed using AutoCad 2007 and Microsoft Office Power Point 2003.

3.6. Printers

In this project two printers were used: Fujifilm Dimatix and Epson Colorio PX 101. The first one is a sophisticated and expensive printer, while the second one is a commercially available and affordable (approximate price of 3000 yen) one.

One of the project goals is to print all the patterns and reagents with EPSON, since the final goal of the project is to create a system as cheap and easy to use as possible. However, at the beginning sensor reagents were printed using Dimatix, although in the final experiments they were printed with EPSON. Regarding the hydrophobic ink used to create the patterns, in most cases it was printed with EPSON, although in some cases Dimatix was used, too.

The back part of the patterns was always printed with EPSON, since Dimatix prints so deeply that the ink crosses all the substrate width, erasing the path designed with the ink.

In *Annex II (Printer settings),* the different settings used in the printers are commented.

4. RESULTS AND DISCUSSION

4.1. *Simple test* patterns
In order to know the basic settings and procedures to print correctly, in the first experiments very simple patterns were printed. These patterns were the “Simple test” series patterns, which basically consisted of two squares and a path connecting them. Almost all these patterns had a 1.5 mm channel width and 0.3 mm wall width. All the pattens designed for this project can be observed at *Annex I (Pattern glossary).*

In figure 6, *Simple test 2* pattern geometry is shown.

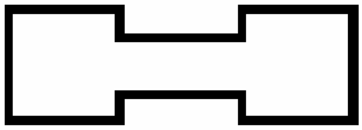


Figure 6. Simple test 2 pattern geometry

In these first experiments, only the front part of the pattern was printed. Dimatix was used. The main printing and ink polymerization settings were:

- Printing configuration: ODADA
- UV light intensity 600 mW
- Polymerization time of 1 min

It must be taken into account that according to previous research done, in order to prevent leaking and path blocking, the minimum wall width was 1 mm and for the channel width was 1.2 mm. However, narrower wall widths were tested in the first experiments realised.

Distilled water was used to test the patterns. The first pattern printed was “Simple test 1”. This pattern presented a channel width of 1.2 mm and a wall width of 0.24 mm. Three patterns were tested. However, although the amount of water dropped in the squares was enough, water did not reach the other side of the channel and got blocked in one of the patterns. In the second one, water got blocked at the entrance of the opposite square. In the last case, approximately 3 minutes were left between the polymerization and the testing, and water was able to flow from one square to the other without problems. In these patterns, leaking from the inner to the outer square were not produced.

In figure 7, a tested *Simple test 1* pattern can be observed.

Figure 7. Simple test 1 pattern tested with red dye. Not real dimensions

Simple test 2, which is almost similar to *Simple test 1* was printed and tested, and no flowing or leaking problems were detected.

Regarding *Simple test 3* patterns, 6 of them were printed. They were printed in series of three, and after they were printed, they were polymerized one by one. So, in the first pattern polymerized, almost no time between printing and polymerization was left, but in the third more than two minutes were left. So, in the first series, the last one could not flow outside the dropping square. In the second series, only the first one flowed properly. One of them showed leaking from the inner to the outer square.

Observing the results, it can be observed that the patterns in which less time was left between printing and polymerization showed better results, wheras the other ones showed bad flowing properties. It seems logical, since ink keeps flowing, spreading, and consequently deforming from the original geometry designed until it is polymerized. So, it can be concluded that the more time is left between printing and polymerization, the worse flowing conditions are obtained.

So, 3 more “Simple test 3” patterns were printed, but this time one by one, so that they could be polymerized just after being printed. The three of them could flow properly from one square to the other. The second one presented leaking, and it was the only that had no time between polymerization and test with distilled water. The other ones had a time lapse of approximately 2 minutes. So, it may be concluded that after polymerization the monomer still requires some time to finish the polymerization and create a proper hydrophobic barrier, which prevents from leaking.

So, in all the next experiments from now on, no time was left between printing and polymerization and at least two minutes were left between polymerization and testing.

Accomplishing the previous settings, some *Simple test* patterns were printed. The difference among those patterns were the square dimensions, since the channel width was 1.2 mm for all of them and for the wall width was 0.3 mm. The pattern testing results are summarized at table 2.

Pattern	Square dimensions (mm^2)	Pattern quantity	Flow Transport	Leaking
Simple test 3	3x3	1	OK	No
Simple test 4	2.5x2.5	4	OK	In one of them
Simple test 5	2x2	4	OK	No
Simple test 6	1.5x1.5	4	OK	No
Simple test 7	1.2x1.2	1	OK	No

Table 2. Simple test patterns series test results

As it can be observed in table 2, when the previously commented times among printing, polymerizing and testing were applied, almost no leaking problems appeared and flow transpost showed good results, which means that water was able to flow from one square to the other.

4.2. Two path patterns

In this section, results obtained using patterns from number 8 to 15 are analysed. These patterns geometry consisted of two areas and two symmetric paths connecting them. As it was told at section *3.5.Geometry of the patterns*, the basic idea was to make the two flows cancel themselves when entering the sensor reagent square, so that the sensor could get wet without spreading throughout the channel and avoiding the coffee ring effect.

4.2.1. Patterns *C_s3w03ch15* and *C_s3cir3w03ch15*

The first patterns created were *C_s3w03ch15* and *C_s3cir3w03ch15*. In figure 8, *C_s3w03ch15* pattern geometry is shown. The main difference between these two patterns is the sensor reagent square geometry. In the first one, it is a square, and in the second one, a circumference. The main reason to create two different geometries was to check if different geometries could lead to different colour change behaviour or to cause differences regarding the coffee ring effect. However, in most of the patterns designed, flow results were not so satisfactory to print the sensor reagent into the pattern, so this difference could not be checked.

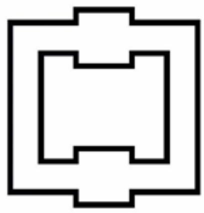


Figure 8. C_s3w03ch15 pattern geometry

All the patterns of this section were printed entirely with EPSON printer using monomer ink with stabilizer. Although in some of *C_s3w03ch15* and *C_s3cir3w03ch15* patterns water flowed through the channels and arrived to the sensor reagent square, in some of them water stopped before arriving. It must be taken into account that in the time between printing and polymerization, the monomer ink spread and deformed a bit, occupying part of the hydrophilic path. So, a feasible explanation of blocking could be the resistance that the monomer ink that invaded the hydrophilic path offered. This made more difficult for water to flow along the path, producing blocking in some cases. Moreover, the path width is narrow, which made easier that blocking occurred.

Another problem detected was the lack of symmetry in the flow speeds. In other words, water did not flow at the same speed on the paths, arriving the analyte at the sensor square at different times. The time difference was so pronounced that in some cases water of one channel reached the sensor reagent square, the square became wet and water continued flowing for the other path until it found the delayed water flow of that path. Obviously, this flowing symmetry problem must be solved in order that the flows cancel themselves and avoid the coffee ring effect.

It is interesting to notice that a lot of water was required to make the flow reach the sensor square, compared with the quantity of water used in the *Simple test* series. This is logic, since *C_s3w03ch15* and *C_s3cir3w03ch15* consist of two paths, and they are longer than the *Simple test* patterns, so more resistance must be surpassed by the analyte.

4.2.2. *shortpath+* pattern series

In order to decrease the path resistance, the *C_s3w03ch15* and *C_s3cir3w03ch15* patterns were modified and paths were shortened. So, “shortpath+” pattern series were designed. These 6 patterns (from patterns 10 to 15) consisted, as the previous ones, of two 3 mm side squares (or a 3 mm diameter circumference in some cases) and a 0.3 mm wall width. 12, 15 and 18 mm path widths were designed depending on the pattern. Polymerization times of 1 and 2 min per every part of the paper were applied.

In figure 9, *C_s3cir3w03ch12_shortpath+* pattern geometry can be observed.

Figure 9. C_s3cir3w03ch12_shortpath+ pattern geometry

At table 3, all the experiments done with the “shortpath+” pattern series can be observed. For each pattern tested, the name and type of the pattern is shown, as well as the date when they were created and tested. It is also shown the time required for the analyte, which in these cases was deionized water, to reach the sensor reagent square for both paths (t_1 cell for the fastest path and t_2 for the slowest) and the time required to get the entire sensor area wet (t sensor area). It must be taken into account that for all the patterns, 5 µl of deionized water were applied.

In the cases that time to get the sensor area wet is empty means that water was blocked or the flow was so slow that vaporized before reaching the sensor reagent area. In the cases that water from one path arrived at the sensor reagent area, filled it and continued for the other path before the other path flow reached the sensor area, only one time to reach the sensor reagent area is shown, since the other path never arrived there. The delay between paths of the same pattern to reach the sensor reagent area (t delay) is shown, too.

Pattern	Name - date	Polymerization time	Leaking	t_1 (s)	t_2 (s)	t delay (s)	t sensor area (s)
C_s3w03ch12_ shortpath+	9-26/5/2011	1 min/pp	No	59	113	54	160
	9'-26/5/2011	1 min/pp	No	51	54	3	151
	9"-26/5/2011	1 min/pp	No	85	118	33	235
	10-26/5/2011	2 min/pp	Yes	146			
	10'-26/5/2011	2 min/pp	Yes				
	10"-26/5/2011	2 min/pp	No	124	235	111	
C_s3w03ch15_ shortpath+	1-2/5/2011	1 min/pp	No	65	87	22	160
	1'-2/5/2011	1 min/pp	No	20	38	18	90
	1"-3/5/2011	1 min/pp	Yes	65	127	62	129
	1'"-3/5/2011	1 min/pp	No	141			
	3'-2/5/2011	2 min/pp	No	83	83	0	220
	3"-3/5/2011	2 min/pp	No	25	36	11	83
	3'"-3/5/2011	2 min/pp	No	44	44	0	110
C_s3w03ch18_ shortpath+	5-3/5/2011	1 min/pp	No	28	35	7	87
	5'-23/5/2011	1 min/pp	No	64	105	41	152
	5"-23/5/2011	1 min/pp	No	28	97	69	119
	6-3/5/2011	2 min/pp	No	21	31	10	73
	6'-23/5/2011	2 min/pp	No	106	106	0	197
	6"-23/5/2011	2 min/pp	No	40	43	3	113
C_s3cir3w03ch12_ shortpath+	11-26/5/2011	1 min/pp	Yes	120			
	11'-26/5/2011	1 min/pp	No	107	131	24	242
	11"-26/5/2011	1 min/pp	No	97	132	35	177
	12-26/5/2011	2 min/pp	No	99	209	110	242
	12'-26/5/2011	2 min/pp	No	129	227	98	372
	12"-26/5/2011	2 min/pp	No	254	318	64	
C_s3cir3w03ch15_ shortpath+	2-2/5/2011	1 min/pp	No	80	310	230	330
	2'-3/5/2011	1 min/pp	Yes	30	54	24	86
	2"-3/5/2011	1 min/pp	Yes	42			
	4-2/5/2011	2 min/pp	No	150	210	60	270
	4'-3/5/2011	2 min/pp	Yes	33	61	28	79
	4"-3/5/2011	2 min/pp	Yes	32	32	0	89
C_s3cir3w03ch18_ shortpath+	7-3/5/2011	1 min/pp	No	38	44	6	93
	7'-23/5/2011	1 min/pp	No	73	132	59	163
	7"-26/5/2011	1 min/pp	No	63	77	14	101
	8-3/5/2011	2 min/pp	No	31	59	28	93
	8'-23/5/2011	2 min/pp	Yes	330	332	2	
	8"-26/5/2011	2 min/pp	No	111	111	0	142

Table 3. shortpath+ patterns series test results

An example of patterns *C_s3cir3w03ch15_ shortpath+* is shown in figure 10.

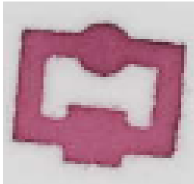


Figure 10. C_s3cir3w03ch15_ shortpath+ pattern tested with red dye. Not real dimensions

Regarding leaking, only a few patterns were affected by it, which is not a serious problem, since as it was commented before, wall width is so thin. Besides, these results are quite positive, since in spite of the thin wall, so little leaking cases occurred, and most of these cases did not present severe leaking. Patterns which suffered leaking had been polymerized for both 1 and 2 minutes, not showing significative differences among them.

Comparing *shortpath+* patterns series and *C_s3w03ch15* and *C_s3cir3w03ch15* peformances, the first ones showed better performances and more reproducible results, with only a few cases of blocking. Besides, *shortpath+* patterns were tested using only 5µl of deionized water, which is a small amount compared with the amount used in *C_s3w03ch15* and *C_s3cir3w03ch15* patterns. So, it was proved that reducing the paths length increased the analyte flow performance.

In order to analyse carefully the previous results, table 4 was done. It shows the average delay and standard deviation between the flow paths of each pattern to reach the sensor reagent area for every type of pattern (independently of the sensor reagent area shape) and type of polymerization. It is also shown the average time needed by the fastest path of every pattern to reach the sensor reagent area. Pattern 2 (made on 2/5/2011) data was not included in the calculations, since its performance was abnormal.

Pattern	**Polymerization time**	**Number of patterns printed**	**Delay time between paths**		**Time to reach the sensor area**	
			Mean (s)	**Standard deviation (s)**	**Mean (s)**	**Standard deviation (s)**
C_s3(cir3)w03ch12_ shortpath+	1 min/pp	6	28.5	21.1	56.3	22.7
	2 min/pp	6	95.8	23.9	160.7	82.2
C_s3(cir3)w03ch15_ shortpath+	1 min/pp	7	31.5	20.5	64.3	35.5
	2 min/pp	6	16.5	24.0	61.2	48.2
C_s3(cir3)w03ch18_ shortpath+	1 min/pp	6	32.7	27.6	49.0	20.0
	2 min/pp	5	6.8	6.4	57.5	36.6

Table 4. shortpath+ patterns series test results classified by path width and polymerization time

Looking at the results, it can be observed that the wider the path channel was, the less delay time and time needed to reach the sensor reagent area were required. It is logic that the wider the path is, the faster it reaches the sensor reagent area, since the ink spread effect is similar in all the patterns, but in the widest path patterns the resistance produced is not appreciated so intensively, as it can be observed in the diagram done in figure 11.

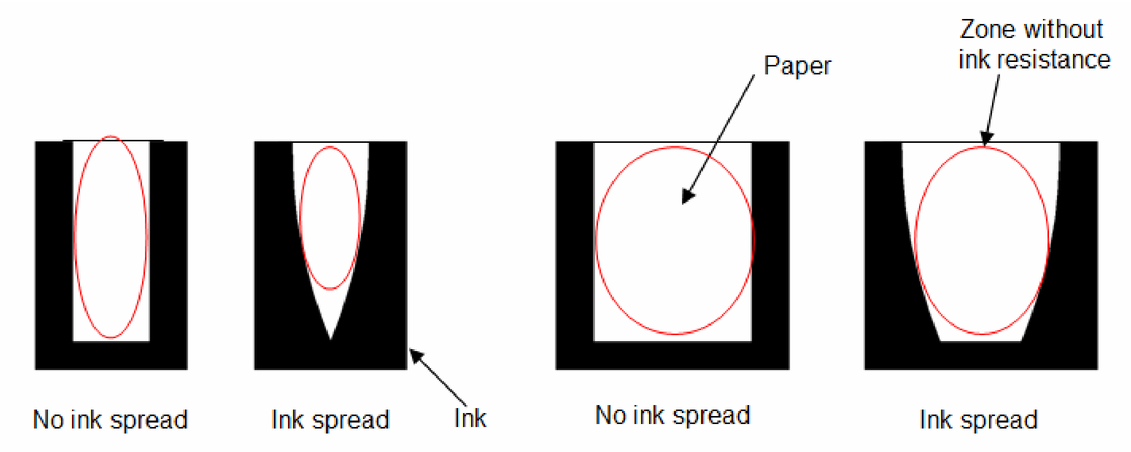


Figure 11. Outline of ink spread and its resistance depending on the path width

It also seems logic that the ink resistance is the main producer of the analyte flow asymmetry, so in wider paths, less asymmetry existed. Although it is true that paper does not possess an homogeneous structure and it probably causes flow asymmetry, it is hydrophilic and the analyte can flow through it, while monomer ink is hydrophobic and the parts of the path invaded by it cannot be crossed by the analyte anymore, producing an important effect in terms of flow asymmetry. Looking at table 4, this fact is checked, since the wider the path is, the less delay between flows occurred.

Regarding the polymerization time, despite time differences could be noticed on the same pattern depending on the polymerization time applied, no relation between the polymerization time and effects in flow velocity and symmetry could be established. It must also be taken into account that experiments reproducibility was very low and standard deviation values confirm it. This poor reproducibility makes difficult to establish empirical relations. Obviously, an important factor that affects seriously the reproducibility of the experiments is the printer used. EPSON is a low cost commercially available printer, and the hydrophobic ink used probably produces partial clogging of the nozzles, leading to differences in the dispensed amounts of ink.

No experiments involving sensor reagents were done, since the performances of the patterns designed was not satisfactory enough.

To check the patterns performance durability made with monomer ink with stabilizer, that needs more polymerization time but has a lower average life than without stabilizer, some patterns were tested again an hour later. In table 5, patterns flow timings observed after polymerization and an hour later can be observed.

Pattern	Name-date	Polymerization time	Leaking	t_1 (s)	t_2 (s)	t delay (s)	t sensor area (s)
C_s3w03ch15_ shortpath+ (after polymerization)	1-2/5/2011	1 min/pp	No	65	87	22	160
	1'-2/5/2011	1 min/pp	No	20	38	18	90
	3'-2/5/2011	2 min/pp	No	83	83	0	220
C_s3w03ch15_ shortpath+ (an hour later)	1-2/5/2011	1 min/pp	No				
	1'-2/5/2011	1 min/pp	No	28	38	10	70
	3'-2/5/2011	2 min/pp	No	40	55	15	165
C_s3cir3w03ch15_ shortpath+ (after polymerization)	2-2/5/2011	1 min/pp	No	80	310	230	330
	4-2/5/2011	2 min/pp	No	150	210	60	270
C_s3cir3w03ch15_ shortpath+ (an hour later)	2-2/5/2011	1 min/pp	No	80			240
	4-2/5/2011	2 min/pp	No	79	170	91	330

Table 5. Effect of time on patterns performance made of monomer ink with stabilizer comparing their flow behaviours just after polymerizing and an hour later

Comparing the five patterns tested it can be observed that in all cases the results changed significantly, partly due to bad reproducibility, and in most of them, patterns performance worsened, becoming the paths blocked in some cases. So, with these experiments it can be concluded that patterns durability using monomer ink with stabilizer is low. This was taken into account in following experiments when the patterns testing was not produced just after polymerization, using in these cases monomer ink without stabilizer.

4.3. Four path patterns

In this section, results obtained using patterns from number 16 to 25 are analysed. Amost all these patterns geometry consisted of two areas connected by two symmetrical paths that bifurcated into two more paths, resulting in four symmetrical paths that entered the sensor reagent area. The idea of designing four paths is to surround the sensor reagent area more effectively than with the two paths patterns, reaching a better flow cancellation and consequently, reducing the coffee ring effect. Obviously, these patterns were designed taking into account the conclusions of the previous section.

4.3.1. Patterns 16 to 21

The first patterns tested was pattern number 16 and 17 (*C_s3cir3w03ch15_shortpath4* and *C_s3w03ch15_shortpath4*), which were printed using EPSON printer and whose geometry can be observed in figure 12. This pattern consisted of a 3 mm side dropping square and 3 mm diameter sensor reagent area. Channel width was 1.5 mm, and the bifurcated paths width was 0.75 mm.

Figure 12. C_s3cir3w03ch15_shortpath4 pattern geometry

Deionized water could not flow through the bifurcated paths, since their width (0.75 mm) was too narrow to let water pass, and water became blocked or it flowed extremely slowly.

Next patterns designed were patterns 18, 19 and 20. The basic difference with the previous ones was a wider path (18 or 30 mm) for both the main paths and the bifurcated ones. The paths geometry was changed to a curved shape, expecting to get better flowing performances than with angular shapes. However, serious leaking and blocking problems appeared, being almost impossible for the flow to reach the sensor reagent area, independently of the deionized water volume applied (10 to 60 μl) or the polymerization time (1 to 4 minutes for every part of the paper).

The cause of these problems was the polymerization method used. The patterns of this section were so wide that the UV light could not surround all the pattern properly, causing an insufficient and inhomogeneous polymerization. So, to polymerize all the next experiments of this section, the UV light was focused alternatively on different parts of the pattern in a symmetric way, in order to assure that all parts of the pattern got polymerized in the same way. An outline of the different ways to polymerize the patterns can be observed in figure 13.

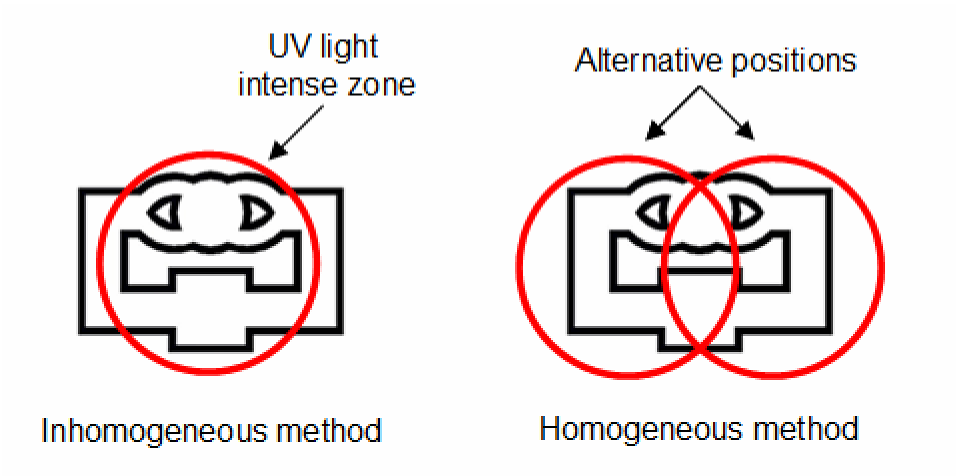


Figure 13. Outline of the different methods to polymerize wide patterns

4.3.2. Pattern *C_s3s4w1ch24_cr_ch04_18_ shortpath4*

To check this polymerization method, pattern number 21 (*C_s3s4w1ch24_cr_ch04_18_ shortpath4*) was printed and tested with 20 µl of both deionized water and red dye.

Polymerization time for every part of the pattern was 3 min, although every part of the pattern was not polymerized for 3 minutes, since as it was told before the UV light focus position was changed alternatively. EPSON printer was used.

In order to have a better and faster polymerization, from this time on in the experiments of this section, monomer ink used had been purified, removing the stabilizer according to the method explained in *3.2.Preparation of the hydrophobic ink*.

In table 6, *C_s3s4w1ch24_cr_ch04_18_ shortpath4* experiments results can be observed. In figure 14, *C_s3s4w1ch24_cr_ch04_18_ shortpath4* pattern geometry and the different pattern zones nomenclature are shown.

Name-date	Test substance	Leaking	tA (s)	tA1 (s)	tA2 (s)	tB (s)	tB1 (s)	tB2 (s)	tC (s)
25-3/6/2011	Dye	No	10	25	25	19			
25'-6/6/2011	Dye	No	2	13	14				42
25''-6/6/2011	Dye	No				16	34	53	97
26-3/6/2011	Deionized water	No	164	390	390	86	312	312	420
26'-6/6/2011	Deionized water	No	20	48	48	96			99
26''-6/6/2011	Deionized water	No	45	153	150	83			390

Table 6. C_s3s4w1ch24_cr_ch04_18_ shortpath4 patterns test results

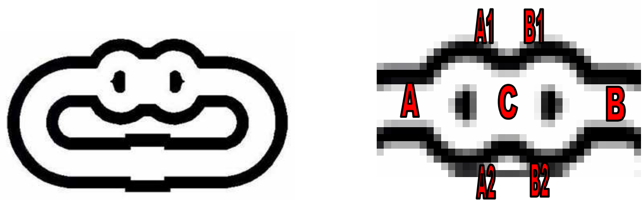


Figure 14. C_s3s4w1ch24_cr_ch04_18_ shortpath4 pattern geometry and its zones nomenclature

Comparing *C_s3s4w1ch24_cr_ch04_18_ shortpath4* and the previous four path patterns polymerized with the inhomogeneous method, it can be observed that pattern performances were clearly better. To begin with, no leaking problems were detected, and blocking and inefficient flow transport problems were reduced considerably. However, in two of the paths analysed, the analyte flowed so slow that did not reach the bifurcation, and in four cases it did not arrive to the sensor reagent area, so this pattern is still very inefficient in terms of analyte flowing.

It is remarkable that in patterns which dye was used as analyte, the flow was considerably faster than the ones which used deionized water. This result is interesting, since it means that some substance properties, such as surface tension, may affect the flowing properties of patterns. So, it would be interesting to do some future research in this field.

In figure 15, pattern 25-3/6/2011 can be observed.

Figure 15. C_s3s4w1ch24_cr_ch04_18_ shortpath4 pattern tested with red dye. Not real dimensions

4.3.3. Pattern *C_s45s4w1ch24_cr_ch04_18_shortpath4*

This pattern design is similar to the previous one, but the dropping square is bigger (4.5 mm), allowing to drop more than 20 µl of analyte. In the next series of experiments, particle based pH sensor reagent, whose synthesizing method is explained at *Abe et al*.[13], was printed into the center of the sensor reagent area.

EPSON printer was used to print the patterns and analyte used was a pH 12 NaOH solution. Polymerization time was 3 min.

The sensor reagent was printed with Dimatix. Cartridge setting used was *pH_indicator_paticle_cartridge*. Sensor reagent geometry was 3x3 mm^2 square, and different printing layers were tested.

In table 7, *C_s45s4w1ch24_cr_ch04_18_ shortpath4* experiments results can be observed.

Name-date	Volume of analyte (µl)	Printing layers	tA (s)	tA1 (s)	tA2 (s)	tB (s)	tB1 (s)	tB2 (s)	tC (s)	Test Observations
29-13/6/2011	30	1	15	21	21	10	15	15	26	No colour remained. Weak colour
30-13/6/2011	20	1	15	22	22				35	Reagent was spread to the right side and bifurcated paths. Weak colour
31-13/6/2011	20	1	49	110	79	48	114	96		Sensor shape was modified. Weak colour
32-14/6/2011	20	10	42			17	29	29		Reagent was spread to the left. Inhomogeneous colour
33-14/6/2011	20	15	24	48	48	40	86	100		Colour change only in some part of the sensor square
34-14/6/2011	30	15	12	19	19					Reagent was spread to the right. Inhomogeneous colour
35-14/6/2011	30	15	15	21	21	23	32	32		Inhomogeneous colour

Table 7. C_s45s4w1ch24_cr_ch04_18_ shortpath4 patterns test results

Figure 16. C_s45s4w1ch24_cr_ch04_18_ shortpath4 pattern geometry and its zones nomenclature

Analysing the results, it can be observed that only one printing layer is such a little amount of sensor reagent, and consequently the colour change obtained was so weak that may not be detected by colour detection software or the quantity used was so little that it was washed by the analyte and disappeared. Otherwise, and independently of the printing layers printed, inhomogeneous colour and sensor reagent spreading occurred, probably due to flow asymmetry. So, these experiments proved that flow asymmetry must be eradicated in order to avoid coffee ring effect and obtain a homogeneous colour change.

Similarly to experiments performed in table 6, pH 12 NaOH solution flowed faster than deionized water solution. Causes of this phenomena may be the same as for dye solution. Moreover, in this case basicity may be an important parameter, too.

No leaking was observed in any of the patterns.

In order to improve patterns performances and avoid the flow asymmetry, more *C_s45s4w1ch24_cr_ch04_18_ shortpath4* were printed using EPSON printer, and different polymerization procedures were applied.

In previous experiments, it was noticed that patterns deformed a bit when they became wet, and this deformation may enhance flow asymmetry. In order to solve this problem, the zone around the patterns was sticked with adhesive tape to the surface where the experiments were being conducted. However, patterns still bent a bit.

To print the patterns, all of them were printed one by one and polymerized, and then tests of the patterns were conducted. So, it must be taken into account that there were time differences between polymerization and tests of the patterns, although they were not significative (no more than 30 minutes). Deionized water was used to perform the tests.

4 polymerization methods were tested:
1) Each side of the paper was polymerized for 3 minutes with 600 mW UV light. On each side of the paper, polymerization was conducted in two zones, changing from one zone to the other every 5s during the first 20s, and changing every 10s from then.
2) Each side of the paper was polymerized for 3 minutes with 600 mW UV light. On each side of the paper, polymerization was conducted in two zones, changing from one zone to the other every 5s.
3) Each side of the paper was polymerized for 6 minutes with 300 mW UV light.
4) Each side of the paper was polymerized for 3 minutes with 300 mW UV light.

In table 8, experiments performed with each method can be observed. Experiments in orange were made and tested with a day difference. *C_s45s4w1ch24_cr_ch04_18_ shortpath4* pattern geometry and its zones nomenclature can be observed at figure 16.

Polymerization method	Name-date	Volume of analyte (μl)	tA (s)	tA1 (s)	tA2 (s)	tB (s)	tB1 (s)	tB2 (s)	tC (s)
1	PT1-16/6/2011	30	308	1080	1080	310	848	780	1200
	PT1'-16//2011	30	440		680	380	820	900	
	PT1d1-21/6/2011	30; after 25' 30μl more	400						
	PT1'd1-21/6/2011	30; after 25' 30μl more	1950			1860			
2	PT1''-16/6/2011	30	33	42	42				69
	PT1'''-16/6/2011	30	270			270			
	PT1''d1-21/6/2011	30; after 25' 30μl more	590	1680	1320	810	1740	1740	1890
	PT1'''d1-21/6/2011	30; after 25' 30μl more				1680			
3	PT2-16/6/2011	30							
	PT2'-16//2011	30	160	400	510	230			
	PT2d1-21/6/2011	30; after 25' 30μl more							
	PT2'd1-21/6/2011	30; after 25' 30μl more							
4	PT3-16/6/2011	30	150	360	360	150	360	360	460
	PT3'-16//2011	30	80	120	120				210
	PT3d1-21/6/2011	30; after 25' 30μl more	420	1860	960				
	PT3'd1-21/6/2011	30; after 25' 30μl more	420	1500	1080	1020	2040	1980	2220

Table 8. C_s45s4w1ch24_cr_ch04_18_ shortpath4 patterns test results using different polymerization methods

The worst results were obtained with method 3), so it may be concluded that an excessive time of polymerization will lead to unsatisfactory performances.

The other polymerization methods showed so dispersed results that proper conclusions could not be made. Results were not reproducible and they showed in some cases extremely asymmetric speed flow results.

The same patterns were made again, and a day between polymerization and test with deionized water was left. Results (which are marked with orange in table 8) showed worse performances and reproducibility than the previous ones. Actually, results were so unsatisfactory that more deionized water had to be dropped after 25 minutes of test time due to the low performances obtained. Although it is possible that time influences pattern performances, these results could be a consequence of the printing conditions, since EPSON printer is a comercially available printer and probably the amount of ink dispensed was changing.

In conclusion, patterns previously designed could not provide symmetric flows, so it is not possible to analyse any analyte parameter with these patterns, since coffee ring effect cannot be avoided.

In order to study more in detail the effect of basic solutions on the patterns, the following experiment was conducted. Patterns PT1d1 and PT2d1, which had been tested with deionized water, were tested with pH 12 NaOH solution once deionized water had been vaporized. And once NaOH solution vaporized, deionized water was applied again. Results can be observed in table 9.

Polymerization method	Name-date	Analyte	Volume of analyte (µl)	tA (s)	tA1 (s)	tA2 (s)	tB (s)	tB1 (s)	tB2 (s)	tC (s)
2	PT1d1-21/6/2011	Deionized water	30; after 25' 30µl more	400						
		NaOH (pH 12)	30	24	34	34	57			50
		Deionized water	30	26	34	34	41			52
3	PT2d1-21/6/2011	Deionized water	30; after 25' 30µl more							
		NaOH (pH 12)	30	30	60	70	70		95	97
		Deionized water	30	86	98	98	91	111	111	115

Table 9. C_s45s4w1ch24_cr_ch04_18_ shortpath4 patterns test results using different analytes

Observing the results obtained, it can be observed that pH 12 NaOH solution flowed extremely faster than the deionized water one. However, when deionized water was dropped again, it flowed as fast as NaOH solution. This may mean that basic solutions can dilute polymer ink contamination that locates in the path or reduce somehow the path resistance. However, it exists the possibility that some NaOH contamination remained in the paper. Future research could be focused on this phenomena understanding.

4.3.4. Pattern *C_s45s4w1ch24_cr_ch04_18_shortpath4_sext9*

Finally, pattern *C_s45s4w1ch24_cr_ch04_18_shortpath4_sext9* was designed. This pattern is similar to the previous one, but an additional 9x9 mm^2 dropping square was made and connected with the previous dropping water square. The basic idea was, on one hand, to create an area to drop a large quantity of analyte in order to surpass the path resistance and on the other hand to drop the analyte in a zone which was not the zone where paths get divided.

If the dropping square is the same square where paths begin, it is possible that flow does not distribute homogeneously in it depending on the exact dropping position, which is clearly influenced by human error, and consequently flowing different water quantities on each path, which will produce flow asymmetry. However, if analyte is dropped in another area, when analyte arrives at the bifurcation area, analyte distribution will be more symmetric. Figure 17 has been created to explain it in more detail.

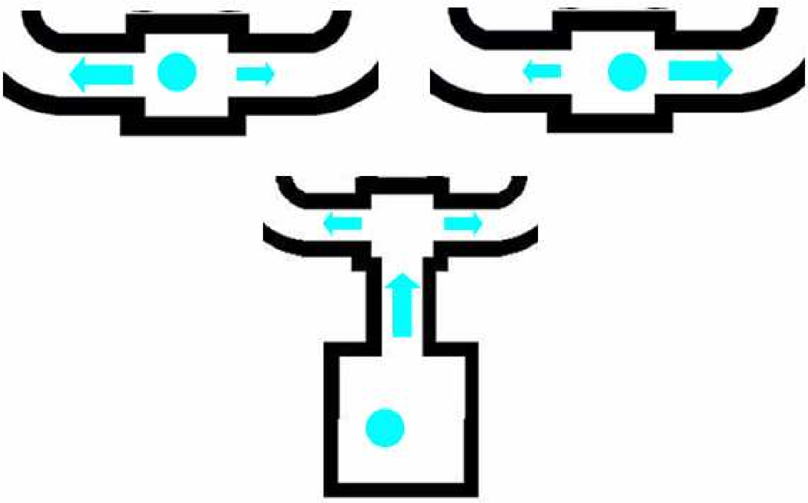


Figure 17. Outline representing analyte distribution depending on pattern geometry

Some patterns *C_s45s4w1ch24_cr_ch04_18_shortpath4_sext9* were tested, but no satisfactory results were achieved. Although a lot of water was dropped (until 400 µl), it remained in the big dropping square and only a little part flowed, so the results obtained were the same as *C_s45s4w1ch24_cr_ch04_18_ shortpath4* results, where blocking, flow asymmetry and low flowing performances occured.

However, the idea of having a dropping square and a separate path bifurcation zone was used in future patterns.

In figure 18, a tested *C_s45s4w1ch24_cr_ch04_18_shortpath4_sext9* pattern can be observed.

Figure 18. C_s3s4w1ch24_cr_ch04_18_ shortpath4 pattern tested with red dye. Not real dimensions

4.4. ***Simple symmetry*** patterns

In order to solve the asymmetry problems, a new series of patterns were made. All the geometry tips learned from the previous sections were applied. For example, the dropping area and the path bifurcation area were located in different zones.

To enhance flow symmetry, patterns designed could be polymerized without changing the UV spotlight position. In other words, all the patterns designed could be located in a 20 mm diameter circumference, which is the UV spotlight diameter. It must be taken into account that changing the UV spotlight position produces polymerization inhomogeneity, since central parts of the pattern get more polymerized than outer parts, and it is not possible to move the spotlight manually locating it in the same position all time. Apart from reducing the patterns size to be polymerized at once, paths were made as big as possible, in order to reduce the ink spread effects in the flow.

This section experiments were performed using monomer ink without stabilizer.

4.4.1. Pattern *Simple symmetry 1*
To begin with, *Simple symmetry 1* pattern was designed. This pattern design is the simplest one, and it is very similar to *Simple test* pattern series, but both dropping, sensor reagent squares and paths dimensions were bigger. In figure 19, *Simple symmetry 1* geometry is shown.

Figure 19. Simple symmetry 1 pattern geometry

In figure 20 a tested *Simple symmetry 1* pattern can be observed.

Figure 20. Simple symmetry 1 pattern tested with red dye. Not real dimensions

The reason to do this pattern was to check that a pattern with the polymerization conditions (without moving the spotlight) and path dimensions commented before would be able to reach satisfactory flow symmetry performances. These patterns were printed with EPSON printer and polymerized for three minutes. 20 μl of deionized water were used to do the tests. Results can be observed at table 10, where time required for the analyte to reach the opposite square is shown.

Name-date	Time to reach the sensor area (s)	Name-date	Time to reach the sensor area (s)
39-23/6/2011	3	44-23/6/2011	135
40-23/6/2011	2	45-23/6/2011	4
41-23/6/2011	9	46-23/6/2011	8
42-23/6/2011	9	47-23/6/2011	69
43-23/6/2011	4		

Table 10. Simple symmetry 1 pattern test result

Although two abnormal results were obtained, the rest were quite similar and reproducible. Once checked this pattern flow performance was satisfactory, two path patterns were designed in order to check the flow symmetry.

4.4.2. Pattern *Simple symmetry 2* and *Simple symmetry 3*
Next pattern designed was *Simple symmetry 2.* This pattern geometry consisted of a dropping square and two sensor reagent squares. The reason to make this pattern was not to print two sensor reagents, but checking the pattern flow symmetry of this kind of pattern.

This pattern test results are shown at table 11. In the test 20 μl of deionized water were used and a polymerization time of 3 minutes was applied.

Name-date	tA (s)	tB (s)	tC (s)	tD (s)
44-23/6/2011	41	41	110	129
45-23/6/2011	34	33	85	93
46-23/6/2011	25	33	75	98

Table 11. Simple symmetry 2 pattern test results

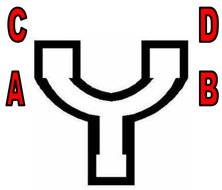


Figure 21. Simple symmetry 2 pattern geometry and its zones nomenclature

In figure 22 a tested *Simple symmetry 1* pattern can be observed.

Figure 22. Simple symmetry 2 pattern tested with red dye. Not real dimensions

As it can be observed, time required to reach the sensor reagent squares was almost similar in the two paths of every pattern, which proves that this new method of doing patterns is able to achieve symmetric results. The average difference time to reach the sensor reagent squares was 3 seconds, which is a quite satisfactory result. Average time difference to fill in the sensor reagent squares was 16.67 seconds. Although this result was not as satisfactory as the previous one, it is a totally acceptable result, and it must be taken into account that sometimes most of the square gets filled in fast, but a small part of it requires more time to be filled. In these results only the time to be completely filled in is only displayed, although in some cases the time difference is not so significative if these no regular areas are not taken into account.

In order to enhance *Simple symmetry 2* pattern performance, *Simple symmetry 3* pattern was designed. In this pattern, sensor reagent squares were closer between them and at the same time, they were closer to the dropping square. Consequently, path length was shortened, and this implies less resistance and a more symmetric flow.

Simple symmetry 3 patterns were printed using both EPSON printer and Dimatix. Some of the patterns were created using only EPSON, and the rest were created using Dimatix to print the front part and EPSON to print the back part. The reason to print the back part with EPSON is that Dimatix prints so deep that ink crosses all paper thickness, so if the back part is printed with Dimatix, ink will fill all the channel created in the front part with ink, erasing it. Two patterns were printed with Dimatix on both sides of the paper. The first one filled all the surface with ink, as it was told before, and in the second one the voltage intensity was reduced in order to avoid this phenomena, but nothing was printed. So, it may be concluded that back parts of the paper cannot be printed with Dimatix and they must be printed with EPSON. So, although Dimatix would be more precise in terms of printing, EPSON inaccuracy cannot be avoided completely

All patterns were polymerized for 3 minutes and tested with 20µl of deionized water. Results are shown in table 12.

Printer	Name-date	tA (s)	tB (s)	tC (s)	tD (s)	∆tA-B	∆tC-D
EPSON	48-23/6/2011	8	11	52	57	3	5
	49-23/6/2011	6	9	49	55	3	6
	50-23/6/2011	9	14	64	72	5	8
	51-23/6/2011	9	11	52	56	2	4
	52-23/6/2011	9	8	62	45	1	17
	53-23/6/2011	10	8	61	65	2	4
	54-23/6/2011	10	9	65	72	1	7
	Average time difference (s)					2.43	6.00
	Standard deviation (s)					1.40	1.58
DIMATIX	56-24/6/2011	5	9	33	42	4	9
	57-24/6/2011	4	6	25	52	2	27
	58-24/6/2011	3	3	23	18	0	5
	59-24/6/2011	4	4	27	27	0	0
	60-24/6/2011	6	6	33	34	0	1
	61-24/6/2011	3	3	13	25	0	12
	Average time difference (s)					1.00	4.50
	Standard deviation (s)					1.67	5.45

Table 12. Simple symmetry 3 pattern test results

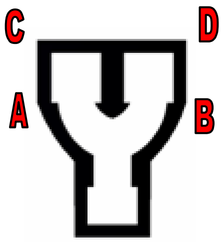


Figure 23. Simple symmetry 3 pattern geometry and its zones nomenclature

Observing the results, ∆tA-B and ∆tC-D were smaller in Dimatix than in EPSON. It can be concluded that Dimatix is more precise than EPSON, although it must be taken into account that back part of Dimatix patterns were printed with EPSON, so EPSON inaccuracy is not avoided completely. Although with Dimatix lower difference time results were achieved, higher standard deviation results were obtained. One of the reasons could be that Dimatix printing times are different depending on the number of nozzles used, and they were sometimes changed from printing to printing . So, Dimatix printing is more precise in terms of printing, producing lower time delay values, but the difference of printing time may produce a wider range of time values in terms of the pattern to be filled with water. However, all results are subjected to experimental errors, and wide range values obtained may be produced by them. In order to reduce the time differences obtained with Dimatix, to use the same nozzles when printing would be a good idea.

∆tC-D average time calculation was done without taking into account results in the patterns 52, 53, 56 and 57, since in these patterns a very small part of one square in every pattern needed more time to be filled, although most of the squares surface needed approximately the same time to be filled. This resulted in a big ∆tC-D value, which does not correspond with reality.

Although Dimatix printer showed better performances, differences were not so significative. So, it has been proved that both printers can be used and very similar results would be obtained.

Comparing *Simple symmetry 2* and *Simple symmetry 3* results, it was proved that reducing the path length decreased the analyte flow time difference to reach the sensor reagent squares, and reproducibility increased.

4.4.3. Pattern *Simple symmetry 4_square*

In previous sections was proved that patterns which are polymerized at once without changing the UV light position and have the bifurcation path zone and the dropping water zone in different zones of the pattern show flow symmetric results. So, using these design tips, *Simple symmetry 4_square* was designed.

Simple symmetry 4_square basically consist of a dropping and a sensor reagent square which are connected by a path that is divided into two symmetrical ones. This pattern geometry, as well as its zones nomenclature, can be observed at figure 24.

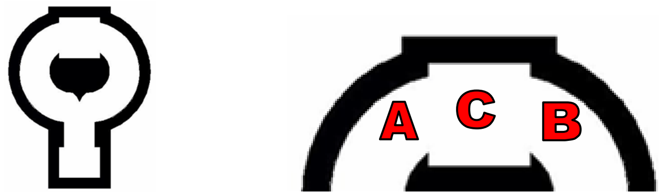


Figure 24. Simple symmetry 4_square pattern geometry and its zones nomenclature

This pattern was polymerized for 3 minutes and tested with 20 µl of deionized water. EPSON printer was used to print them. Results can be observed at table 13.

Name-date	Volume of analyte (µl)	tA (s)	tB (s)	∆tA-B	tC (s)
67-30/6/2011	20	115	170	55	250
68-30/6/2011	20	131	235	104	327
69-30/6/2011	20	230	230	0	370
	Average time difference (s)			53.00	
	Standard deviation (s)			52.03	
67-30/6/2011	30	65	55	10	134
68-30/6/2011	30	57	69	12	130
69-30/6/2011	30	75	103	28	182
	Average time difference (s)			16.67	
	Standard deviation (s)			9.87	

Table 13. Simple symmetry 4_square pattern test results

Looking at the results obtained, an important average time difference between paths increment can be observed compared with *Simple symmetry 3* pattern. Observing the results, it can be concluded that slight increment of path length produce an important increment of path resistance, and consequently, more time is required to cross the path and flow asymmetry grows. An important flow asymmetry decrease and time required to cross the path reduction is produced when analyte quantity is slightly increased, as it can be observed in table 13. In other words, pattern symmetry is very sensitive to small changes of the system.

In figure 25, pattern *Simple symmetry 4_square* can be observed.

Figure 25. Simple symmetry 4_square pattern tested with red dye. Not real dimensions

4.4.4. Pattern *Simple symmetry 4* and *Simple symmetry 5*

In this section a new concept of pattern was tested. All the patterns previousy designed had a dropping square where analyte was dropped. Having a limited surface to drop the analyte implies that the analyte quantity dropped is limited, and this may be a problem in some cases.

In order to avoid this limitation, pattern *Simple symmetry 4* was designed. This pattern was similar to *Simple symmetry 4_square*, but the dropping zone was substituted by an opened area. To use this pattern, it was submerged vertically into the analyte until the opened zone was in contact with the analyte. Then, the analyte flowed through the pattern, but no analyte quantity limitation existed.

In figure 26, *Simple symmetry 4* pattern geometry, as well as its zones nomenclature, can be observed.

Figure 26. Simple symmetry 4 pattern geometry and its zones nomenclature

The first *Simple symmetry 4* patterns were printed with EPSON and each part of the paper was polymerized for 3 minutes. Tests were performed with distilled water.

Name-date	tA (s)	tB (s)	∆tA-B	tC (s)
62-28/6/2011	95	100	5	79
63-28/6/2011	215	240	25	275
64-28/6/2011	265	300	35	375
65-28/6/2011	187	201	14	260
66-28/6/2011	114	131	17	167
	Average time difference (s)		19.20	
	Standard deviation (s)		11.37	

Table 14. Simple symmetry 4 pattern test results

As it can be observed, results obtained were very similar to *Simple symmetry 4_square* patterns when 30 µl were used, so the pattern performance can be considered satisfactory. However, the outer zone of the pattern became completely wet with analyte, and risk of leaking, from both outside to inside the pattern and vice versa, existed.

In the next series of experiments *Simple symmetry 4* pattern was printed again, and particle based pH sensor reagent was printed, too.

As it was shown in 4.4.2 (Pattern *Simple symmetry 2* and *Simple symmetry 3)*, EPSON and Dimatix performances were almost similar in terms of flow asymmetry. So, results obtained with both printers would be similar. However, for these first experiments with sensor reagents, pattern performance was considered a critical parameter, and Dimatix printer was used to print the front part of the patterns. Obviously, once the pattern performances and sensor printing procedures have been optimized, EPSON printer would be used again to print both parts of the patterns.

The pH sensor reagent was printed with Dimatix. The analyte used was a pH 8 solution. Polymerization time was 3 minutes for every part of the paper.

Results of the experiments can be observed in table 15. It must be taken into account that the time needed to fill in the sensor reagent cannot be written down when sensor reagents are printed, since it is not possible to observe when the sensor reagent has become completely wet.

Name-date	**Sensor printing layers**	**Sensor square dimensions (mm^2)**	**tA (s)**	**tB (s)**	**∆tA-B**
70-30/6/2011	15	3x3	450	450	0
71-30/6/2011	10	3x3	87	120	33
72-30/6/2011	10	3x3	160	160	0
73-30/6/2011	10 (interlayer time 40s)	3x3	300	300	0
74-30/6/2011	10 (interlayer time 60s)	2x2	330	330	0
		Average time difference (s)			6.60
		Standard deviation (s)			14.76

Table 15. Simple symmetry 4 pattern test results using particle based pH sensor

Surprisingly, flow symmetry considerably improved. Although this improvement could be influenced by the use of Dimatix printer, so much time difference between the previous series of experiments and these series is a bit anomalous.

Regarding the pH sensor reagent, in most patterns colour change was weak and a lot of time was required to notice it. The amount of sensor reagent printed is not so high, and actually it was reduced by making the sensor reagent square smaller and reducing the number of printing layers, so a feasible explanation may be that only a little analyte arrived at the sensor reagent area. If this hypothesis is correct, it will mean that the analyte cannot flow through the channels properly due to the effect of gravity, and it may be possible, since the time required to arrive to the sensor reagent area in the patterns that were disposed vertically was higher. Otherwise, so many minutes after the test, the sensor reagent square disappeared. This was caused by leaking. There was so much analyte in the outer zone of the pattern that analyte flowed from outside to inside, and this analyte washed away and diluted the sensor reagent.

When printing the pH sensor reagent, it was noticed that the first printing layers were printed properly, but from the fifth one, approximately, the square was not uniform anymore and the following printing layers spread a bit outside the square. This was produced because the sensor reagent did not have enough time to vaporize before the next printing layer began to be printed. Consequently, some liquid remained in the square and produced leaking, spreading the sensor reagent outside the square. In order to avoid this effect, some interlayer time was left between printing layers. In the last patterns of this series, 40 and 60 seconds were left. However, some square disuniformity still remained.

In order to solve the leaking problems, *Simple symmetry 5* pattern was designed. This pattern is like *Simple symmetry 4*, but a wider wall was designed in order to prevent leaking. *Simple symmetry 5* geometry can be observed in figure 27.

Figure 27. Simple symmetry 5 pattern geometry and its zones nomenclature

The same printing, reagents and polymerization conditions than in *Simple symmetry 4* experiments were applied, except interlayer printing time, which was increased to avoid the sensor reagent square disuniformity.

Patterns 75 to 78 were printed and tested. As *Simple simmetry 4* patterns, colour change was almost no noticed. Leaking did not occur, so making the wall width wider was useful.

In conclusion, these patterns that consisted in letting as much as analyte as possible to enter the pattern did not show good performances due to the gravity effect. Besides, if the wall width was not wide enough, leaking occurred and washed away the sensor reagent. So, these patterns did not result to be useful for the project.

4.4.5. Pattern *Simple symmetry 6*

4.4.5.1. Test using EPSON and Dimatix

This pattern is a *Simple symmetry 4_square* pattern improved. The dropping square zone was increased, in order to allow more analyte to be dropped, and the path was widened to 4 mm. In figure 28, *Simple symmetry 6* geometry can be observed.

Figure 28. Simple symmetry 6 pattern geometry

Patterns of this section were printed using EPSON for the back part of the paper and Dimatix for the front part. The different sensor reagents used were printed with Dimatix, too. Polymerization time was 3 minutes for each side of the paper.

Avoiding the coffee ring effect using particle based pH sensor reagents is a goal that has been achieved yet. So, with this pattern non particle based pH sensor reagent solution was used to prove that the sensing system studied works correctly with the two types of solution. It must be taken into account that unlike particle based pH sensor reagent solution, the non particle one does not get sticked to the substrate and it spreads and it is affected by coffee ring effect easily, so working with it supposes a greater challenge than the non particle one. Non particle based pH indicator printing configuration was *pH_indicator* (more details explained at *Annex II. Printing settings*).

In all the patterns done, 15 layers of analyte were printed. The shape selected was 1x1 mm^2 square, since its dimensions are small enough to assure that there was enough analyte to react with all the sensor reagent. Besides, in order to assure no sensor reagent displacement, it is better the analyte flow to surround completely the sensor reagent square, so square size (1x1 mm^2) must be smaller than flow path width (4 mm). Interlayer printing time was 75s, which was sufficient time to assure a homogeneous sensor reagent distribution.

Some pH buffer solutions, from pH 5 to 9, were done and they were used as analytes. After testing all these buffer solutions, a calibration curve was created in order to check if it is feasible to measure analytes properties with the system studied in this project. The procedure to create the different pH buffer solutions used are specified at section 3.4 (Preparation of the pH buffers). The analyte amount used in the patterns was 12 µl, except in pattern 106, that needed 23µl.

Before and after doing the patterns tests, they were scanned in order to detect the sensor reagent colour digitally (Digital Color Meter software Ver. 3.6.1 provided with the Mac OS X Ver. 10.5). The colour detection system used was the L*a*b* system, and the parameter used to analyse the colour difference was a*, since the most important colour change occurred in the red tonality. Moreover, in order to check the relation between colour change and time, patterns were scanned before testing and at different times after testing. In *Annex III. Simple symmetry 6 (made with EPSON and Dimatix) patterns using non particle pH based sensor reagent. Colour and image data* patterns colour data, as well as their images, can be observed.

Observing all patterns colour data before being tested, it can be noticed that the printing reproducibility was so high, and the initial a* values were very similar, being the average value 15.782 and the standard deviation 0.671. So, in order to analyse the colour change, only the testing a* value must be taken into account, since the initial a* value was approximately the same for all the patterns.

Looking at the results, it can also be observed that the colour values depended on time. The most reproducible and coherent results were obtained, approximately, between 10 and 25 minutes after the test. It is logical that some time is required to let the flow reach all the analyte layers and that after some time the pH sensor reagent may be affected by other factors that may interfere in the colour change, such as its dilution into the analyte. Moreover, depending on the pH buffer, different times were required to change the colour, so, although the optimal colour data was obtained between 10 and 25 minutes, depending on the case these time lapse may be slightly modified.

In figure 29, a calibration curve relating the colour and the pH can be observed. As it was commented previously, the values used to do it were taken between 10 and 25 minutes after the test.

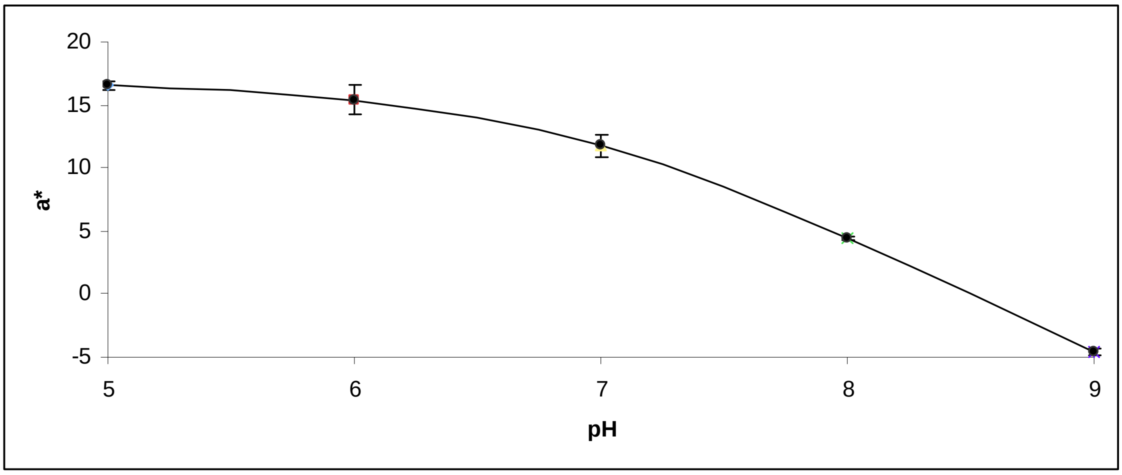


Figure 29. Calibration curve correlating the colour data obtained using pH buffer solutions in Simple symmetry 6 patterns

A good calibration curve with so little error could be created. The error bars indicate the samples standard deviation values (4 samples were performed for every pH buffer). However, it must be taken into account that only two of the four experiments made with pH 7 buffer were used, since the rest showed very different values.

Regarding coffee ring effect, it could be avoided in a great quantity of the patterns tested. However, some of them showed some, since the flow symmetry obtained was not perfect, pushing away the sensor reagent in some cases. This is not a serious problem, since the coffee ring effect produced in these cases was not pronounced at all and it did not affect the results.

In conclusion, the creation of this calibration curve demonstrates that two paths patterns are a feasible method to detect analyte properties, avoiding to a large degree the coffee ring effect and providing so exact results.

4.4.5.2. Test using only EPSON

Once it was demonstrated that two path channels are a feasible and useful tool, the same experiment was done again, in the same conditions, but this time using only EPSON printer. In other words, the sensor reagent was printed using EPSON printer.

The sensing reagent zone dimensions were the same than in the previous experiment, as well as the interlayer printing time. However, the number of layers printed was changed to 10 layers, instead of 15.

As it can be observed in *Annex IV. Simple symmetry 6 (made only with EPSON) patterns using non particle pH based sensor reagent. Colour and image data*, EPSON results showed a good reproducibility. For example, the standard deviation value of the sensor reagent colour data (a*) before being tested was 0.97, which is similar to the result obtained when Dimatix was used in the previous experiment (0.67). Moreover, colour data values were almost similar, being 15.78 when Dimatix was used and 15.09 when EPSON was used.

In figure 30, the calibration curve obtained with the different pH values can be observed.

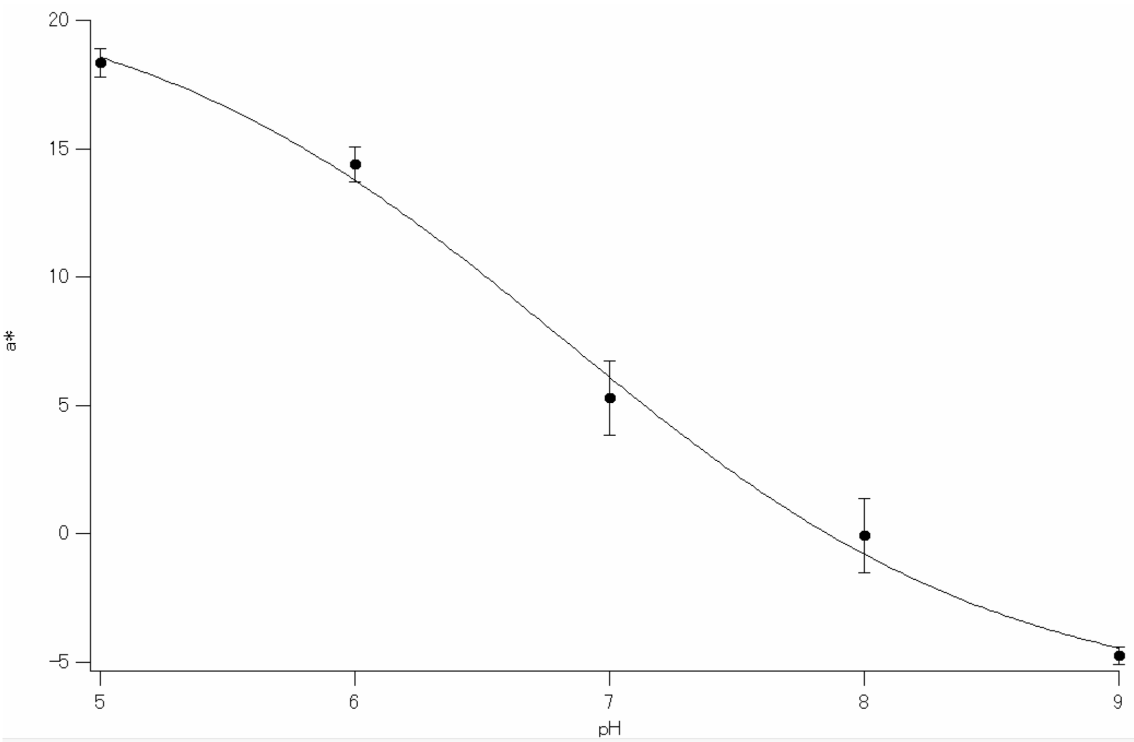


Figure 30. Calibration curve correlating the colour data obtained using pH buffer solutions in Simple symmetry 6 patterns. Only EPSON printer was used.

As it can be observed, results were so reproducible, and standard deviation values were acceptable in all cases, although in pH 7 and 8 the standard deviation values were higher than in the previous experiment. It must be taken into account that in some cases coffee ring effect appeared, as it can be observed in figure AIV6 of *Annex IV. Simple symmetry 6 (made only with EPSON) patterns using non particle pH based sensor reagent. Colour and image data*, and in some of these cases the colour detection zone was moved from the original sensing reagent zone, in order to achieve more reliable results.

It must be taken into account that for this experiment new non particle based pH sensor reagent was done, so colour data obtained for the different pHs varied from the ones obtained in the previous experiment.

In conclusion, in this last experiment it was demonstrated that it is possible to detect quantitatively a substance property, like pH, using only a commercially available printer to print the reagents, making more feasible the possibility of using and creating this kind of sensing system in the same place where it is required. Although in this case the sensor reagent used was a pH sensor reagent, it could be used with other kinds of sensor reagents, too.

5. Conclusions

The main goal of this project was to develop a sensing system that was able to detect analytes or analyte properties without the necessity of either qualified professionals or sophisticated equipment. This goal has been achieved, since a sensing device was created using a commercially available printer, an UV light, a scanner and a simple colour detection software. The system designed was proved using a particle and a non particle based pH sensing reagent, and for both of them a calibration curve relating pH values and sensor reagent colour data were perfomed. Moreover, the system designed is not only limited to pH sensor reagents, and more sensor reagents may be used, since the system is versatile, accepting even the use of aqueous (non particle based) reagents, too.

A better comprehension of the ink spread effect and its consequences was obtained. Ink spread was the main cause of assymetry in the patterns, since hydrophobic ink invades the hydrophilic path in an irregular way, producing irregular flow resistance, which is increased enormously when the path is lengthened. So, interesting pattern design tips, such as the most suitable polymerization conditions, were learned in order to avoid assymetry and path resistance.

It was also demonstrated that two path and four path channels are feasible methods to reduce flow asymmetry, and consequently the coffee ring effect produced when the analyte washed away the sensor reagent.

6. Future research

In this project a sensing system was presented, but it was only tested with pH sensing reagent. However, one of the project aims was to create a sensing device as universal as possible, so more sensing reagents should be tested.

Patterns design could be improved in order to improve patterns performances. For example, a four path channel may be designed using the *Simple symmetry* patterns design criteria. This new design may be able to reduce the coffee ring effect.

It was observed that depending on the analyte used, flow velocity changed. It was also observed that the more basic or acid the analyte pH was, the faster it flowed, while the more neutral, the slower it flowed. It would be interesting to check which are the factors that produce this phenomena, as well as checking if the paper structure was modified.

Finally, it would be interesting to modify the patterns created in order to create multianalyte sensing devices, such the one showed in figure 31.

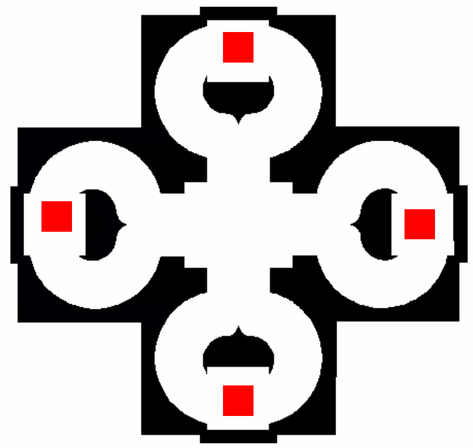


Figure 31. Multianalyte sensing pattern designed using the Simple symmetry patterns design criteria

7. Acknowledgement

I would like to thank Daniel Citterio (Master's Thesis advisor), Imoto Shota and Maejima Kento for the assistance and advice provided, and to Suzuki Koji for letting me to do the project in the laboratory he directs.

8. Bibliography

[1] Martinez, A.W. *et al*. *Diagnostics for the developing world: microfluidic paper-based analytical devices*. Analytical Chemistry 82(1), 2010.

[2] Martinez A.W. *et al*. *Flash: a rapid method for prototyping paper-based microfluidic devices*. Lab on a Chip [www.rsc.org/loc].

[3] Martinez A.W. *et al*. *Three-dimensioanl microfluidic devices fabicated in layered paper and tape*. Proc. Natl. Sci. U.S.A. 105, 2008.

[4] Carrilho E. et al. *Understanding wax printing: a simple micropatterning process for paper-based microfluidics*. Analytical Chemistry 81(16), 2009.

[5] Sele, C. W. *et al*. Self-*aligned inkjet printing with sub-hundred nanometer resolution*. Adv. Mater. 17, 2005.

[6] Sirringhaus, H. *et al*. *High-resolution ink-jet printing of all-polymer transistor circuits*. Science 290, 2000.

[7] de Gans, B. *et al*. *Inkjet printing of polymers: State of the art and future developments*. Advanced Materials, 16(3), 2004.

[8] Yoshioka Y. *et al*. *Simple modification of sheet resistivity of conducting polymeric anodes via combinatorial ink-jet printing techniques.* Macromolecular Rapid Communications, 26(4), 2005.

[9] Setti L. *et al. An amperometric glucose biosensor prototype fabricated by thermal inkjet printing*. Biosensors and Bioelectronics, 20(10), 2005.

[10] Setti L. *et al. An HRP-based amperometric biosensor fabricated by thermal ink-jet printing.* Sensors and Actuators, 126(1), 2007.

[11] Abe K. et *al. Inkjet-printed paperfluidic immuno-chemical sensing device.* Analytical and bioanalytical chemistry, 398(2), 2010.

[12] Abe K. *et al. Inkjet-Printed Microfluidic Multianalyte Chemical Sensing Paper.* Analytical Chemistry, 80(18), 2008.

[13] Fenton E. M. *et al. Multiplex lateral-flow test strips fabricated by two-dimensional shaping*. Applied Materials & Interfaces. 1(1), 2009.

[14] Bruzewicz D. A*. et al. Low-cost printing of poly(dimethylsiloxane) barriers to define microchannels in paper*. Analytical Chemistry, 80(9), 2008.

[15] Martinez A.W. *et al. Diagnostics for the developing world: microfluidic paper-based analytical devices*. Analytical Chemistry, 82(1), 2010.

[16] *Lab on paper*. Lab on a Chip, 8, 2008.

[17] Aikio S. *et al. Bioactive Papers and Fiber Products: Patent and Literatura Survey*. VTT Working Papers, 51, 2006.

[18] Zimmermann M. et *al. Capillary pumps for autonomous capillary systems*. Lab on a Chip, 7(1), 2007.

[19] Martinez A.W. *et al. Patterned paper as a platform for inexpensive, low-volume, portable bioassays*. Andgewandte Chemie International Edition, 46(8), 2007.

[20] Bruzewicz D.A.*et al. Low-cost printing of poly(dimethylsiloxane) barriers to define microchannels in paper*. Analytical Chemistry, 80(9), 2008.

[21] Martinez A.W. *et al. Simple telemedicine for developing regions: camera phones and paper-based microfluidic devices for realtime, off-site diagnosis*. Analytical Chemistry, 80(10), 2008.

[22] Su S.*et al. Microgel-based inks for paper-supported biosensing applications*. Biomacromolecules, 9(3), 2008.

ANNEX

Annex. Index

I. Pattern glossary

In this section a glossary with all the patterns designed for the project has been done. All patterns shapes can be seen observed together with their respective names and important measures.

In most cases, pattern names provide useful information about them. For example, “Simple test” pattern series refer to the patterns made at the beginning of the project to establish some basic geometry measures, and “Simple symmetry” patterns refer to the last patterns done, which, were very simple in order to solve their symmetry problems.

However, most of the pattern names provide information about their geometry and measures. The standard name may be:

C_sX1sX2cirX3wX4chX5 (1)

- sX1 indicates the dropping square side length
- sX2 indicates the sensor square side length, in case it would be different from the dropping square one
- cirX3 indicates the sensor zone diameter, in case its shape would be a circumference
- wX4 indicates the pattern wall width
- chX5 indicates the channel width

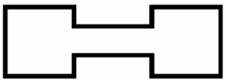
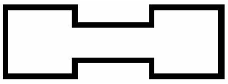
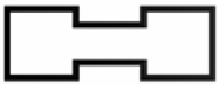
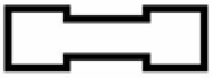
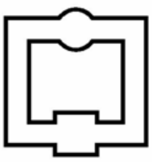
It must be taken into account that all measures are given in mm (decimal points were omitted). Pattern names endings provide information, too. For example, “_shortpath+” refers to the patterns whose path was made shorter in order to enhance their performance. “_shortpath4” refers to patterns whose channels are divide into two.

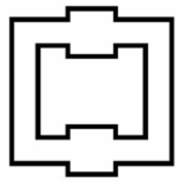
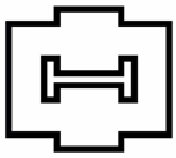
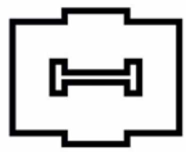
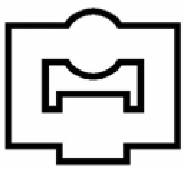
In the case of “_shortpath4” patterns, their name may be lengthened in order to provide more useful information. A standard name may be:

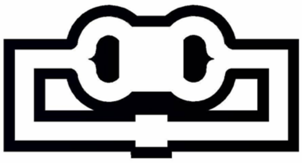
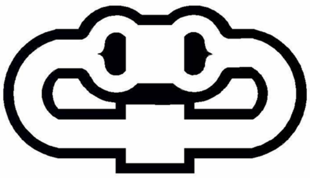
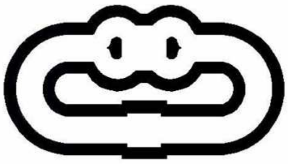
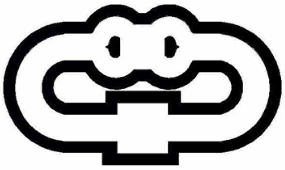
C_sX1sX2cirX3wX4chX5_cr_ch04_X6_shortpath4 (2)

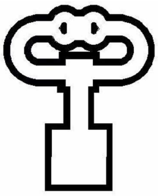
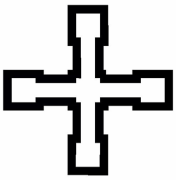
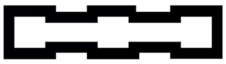
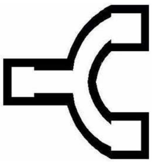
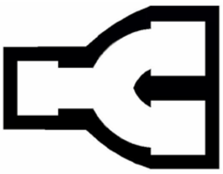
- _cr indicates that the path between the dropping square and the sensor zone has a bent shape. If it is not the case, _cr does not appear
- _ch04_ X6 indicates path width of the bifurcated channels

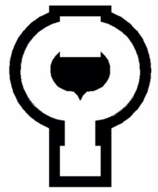
For example, C_s45s4w1ch24_cr_ch04_18_shortpath4 indicates that the pattern is composed by two squares, the dropping one has a side of 4.5mm and the sensor one of 4 mm. The channel width is 1 mm and the path width is 2.4 mm. The paths are bifurcated in two, and each has a width of 1.8 mm. The path between the two squares has a bent shape.

1		Name: Simple test 1	
		Dropping square: 3.2x3.2 mm^2	Channel width: 1.12 mm
		Sensor square: 3.2x3.2 mm^2	Wall width: 0.24 mm
		Other:	
2		Name: Simple test 2	
		Dropping square: 3.2x3.2 mm^2	Channel width: 1.5 mm
		Sensor square: 3.2x3.2 mm^2	Wall width: 0.3 mm
		Other:	
3		Name: Simple test 3	
		Dropping square: 3x3 mm^2	Channel width: 1.5 mm
		Sensor square: 3x3 mm^2	Wall width: 0.3 mm
		Other:	
4		Name: Simple test 4	
		Dropping square: 2.5x2.5 mm^2	Channel width: 1.5 mm
		Sensor square: 2.5x2.5 mm^2	Wall width: 0.3 mm
		Other:	
5		Name: Simple test 5	
		Dropping square: 2x2 mm^2	Channel width: 1.5 mm
		Sensor square: 2x2 mm^2	Wall width: 0.3 mm
		Other:	
6		Name: Simple test 6	
		Dropping square: 1.5x1.5 mm^2	Channel width: 1.5 mm
		Sensor square: 1.5x1.5 mm^2	Wall width: 0.3 mm
		Other:	
7		Name: Simple test 7	
		Dropping square: 1.2x1.2 mm^2	Channel width: 1.5 mm
		Sensor square: 1.2x1.2 mm^2	Wall width: 0.3 mm
		Other:	
8		Name: C_s3cir3w03ch15	
		Dropping square: 3x3 mm^2	Channel width: 1.5 mm
		Sensor area: 3 ϕmm	Wall width: 0.3 mm
		Other:	

9		Name: C_s3w03ch15	
		Dropping square: 3x3 mm^2	Channel width: 1.5 mm
		Sensor square: 3x3 mm^2	Wall width: 0.3 mm
		Other:	
10		Name: C_s3w03ch12_shortpath+	
		Dropping square: 3x3 mm^2	Channel width: 1.2 mm
		Sensor square: 3x3 mm^2	Wall width: 0.3 mm
		Other:	
11		Name: C_s3w03ch15_shortpath+	
		Dropping square: 3x3 mm^2	Channel width: 1.5 mm
		Sensor square: 3x3 mm^2	Wall width: 0.3 mm
		Other:	
12		Name: C_s3w03ch18_shortpath+	
		Dropping square: 3x3 mm^2	Channel width: 1.8 mm
		Sensor square: 3x3 mm^2	Wall width: 0.3 mm
		Other:	
13		Name: C_s3cir3w03ch12_shortpath+	
		Dropping square: 3x3 mm^2	Channel width: 1.2 mm
		Sensor area: 3 ϕmm	Wall width: 0.3 mm
		Other:	
14		Name: C_s3cir3w03ch15_shortpath+	
		Dropping square: 3x3 mm^2	Channel width: 1.5 mm
		Sensor area: 3 ϕmm	Wall width: 0.3 mm
		Other:	
15		Name: C_s3cir3w03ch18_shortpath+	
		Dropping square: 3x3 mm^2	Channel width: 1.8 mm
		Sensor area: 3 ϕmm	Wall width: 0.3 mm
		Other:	
16		Name: C_s3cir3w03ch15_shortpath4	
		Dropping square: 3x3 mm^2	Channel width: 1.5 mm
		Sensor area: 3 ϕmm	Wall width: 0.3 mm
		Other: bifurcated paths were approximately 0.75 mm	

17		Name: C_s3w03ch15_shortpath4	
		Dropping square: 3x3 mm^2	Channel width: 1.5 mm
		Sensor square: 3x3 mm^2	Wall width: 0.3 mm
		Other: bifurcated paths width was approximately 0.75 mm	
18		Name: C_s3s6w1ch18ch04_18_shortpath4	
		Dropping square: 3x3 mm^2	Channel width: 1.8 mm
		Sensor square: 6x6 mm^2	Wall width: 1 mm
		Other: bifurcated paths width was 1.8 mm	
19		Name: C_s6w1ch18_cr_ch04_18_shortpath4	
		Dropping square: 6x6 mm^2	Channel width: 1.8 mm
		Sensor square: 6x6 mm^2	Wall width: 1 mm
		Other: bifurcated paths width was 1.8 mm	
20		Name: C_s6w1ch30_cr_ch04_18_shortpath4	
		Dropping square: 6x6 mm^2	Channel width: 3 mm
		Sensor square: 6x6 mm^2	Wall width: 1 mm
		Other: bifurcated paths width was 1.8 mm	
21		Name: C_s3s4w1ch24_cr_ch04_18_shortpath4	
		Dropping square: 3x3 mm^2	Channel width: 2.4 mm
		Sensor square: 4x4 mm^2	Wall width: 1 mm
		Other: bifurcated paths width was 1.8 mm	
22		Name: C_s45s4w1ch24_cr_ch04_18_shortpath4	
		Dropping square: 4.5x4.5 mm^2	Channel width: 2.4 mm
		Sensor square: 4x4 mm^2	Wall width: 1 mm
		Other: bifurcated paths width was 1.8 mm	
23		Name: C_s3w1ch18_shortpath+	
		Dropping square: 3x3 mm^2	Channel width: 1.8 mm
		Sensor square: 3x3 mm^2	Wall width: 1 mm
		Other:	
24		Name: C_s3cir3w1ch18_shortpath+	
		Dropping area: 3x3 mm^2	Channel width: 1.8 mm
		Sensor square: 3 ϕmm	Wall width: 1 mm
		Other:	

25		Name: C_s45s4w1ch24_cr_ch04_18_shortpath4_sext9	
		Dropping square: 4.5x4.5 mm^2	Channel width: 2.4 mm
		Sensor square: 4x4 mm^2	Wall width: 1 mm
		Other: bifurcated paths width 1.8 mm, external square 9x9 mm^2	
26		Name: C_s3long5_04w1ch18	
		Dropping square: 3x3 mm^2	Channel width: 1.8 mm
		Sensor square: 3x3 mm^2	Wall width: 1 mm
		Other: square distance 5 mm	
27		Name: C_s3long5_02w1ch18	
		Dropping square: 3x3 mm^2	Channel width: 1.8 mm
		Sensor square: 3x3 mm^2	Wall width: 1 mm
		Other: square distance 5 mm	
28		Name: C_s3long6s3long5w1ch18	
		Dropping square: 3x3 mm^2	Channel width: 1.8 mm
		Sensor square: 3x3 mm^2	Wall width: 1 mm
		Other: square distance are 6 and 5 mm (from left to right)	
29		Name: Simple symmetry 1	
		Dropping square: 3x4 mm^2	Channel width: 3 mm
		Sensor square: 3x4 mm^2	Wall width: 1 mm
		Other:	
30		Name: Simple symmetry 2	
		Dropping square(left): 3x4 mm^2	Channel width: 3 mm
		Sensor square: 4x4 mm^2	Wall width: 1 mm
		Other:	
31		Name: Simple symmetry 3	
		Dropping square(left): 3x4 mm^2	Channel width: 3 mm
		Sensor square: 4x4 mm^2	Wall width: 1 mm
		Other:	
32		Name: Simple symmetry 4	
		Dropping square:	Channel width: 3 mm
		Sensor square: 4x4 mm^2	Wall width: 1 mm
		Other:	

33		Name: Simple symmetry 4_square	
		Dropping square: 4x3 mm^2	Channel width: 3 mm
		Sensor square: 4x4 mm^2	Wall width: 1 mm
		Other:	
34		Name: Simple symmetry 5	
		Dropping square:	Channel width: 3 mm
		Sensor square: 4x4 mm^2	Wall width: 1-5 mm
		Other:	
35		Name: Simple symmetry 6	
		Dropping square:7.87x2.85mm^2	Channel width: 4 mm
		Sensor square: 5x5 mm^2	Wall width ≥ 1 mm
		Other:	

II. Printer settings

II.1. Dimatix settings

II.1.1. ODADA

- Reagent printed: ODADDA hydrophobic ink
- Printheads voltage: 22 V
- Drop spacing: 20 µm
- Cartridge print height: 0.850 mm
- Cleaning cycle: spit_purge0.1sec_spit
- Jetting waveform:

Figure AII1. ODADA setting configuration jetting waveform

II.1.2. pH_indicator_particle

- Reagent printed: particle based pH sensing reagents
- Printheads voltage: 26 V
- Drop spacing: 20 µm
- Cartridge print height: 0.850 mm
- Cleaning cycle: spit_purge0.1sec_spit
- Jetting waveform:

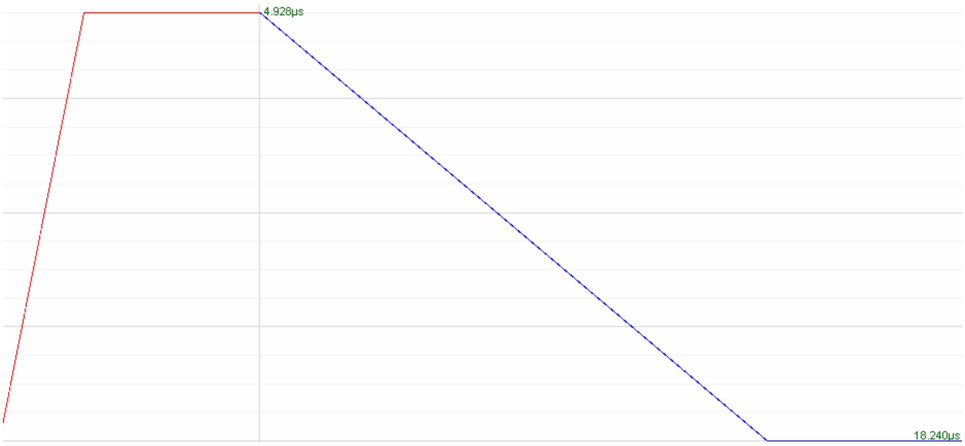


Figure AII2. pH_indicator_particle *setting configuration jetting waveform*

II.1.3. pH_indicator

- Reagent printed: non particle based pH sensing reagents
- Printheads voltage: 22 V
- Drop spacing: 20 µm
- Cartridge print height: 0.850 mm
- Cleaning cycle: spit_purge0.1sec_spit
- Jetting waveform:

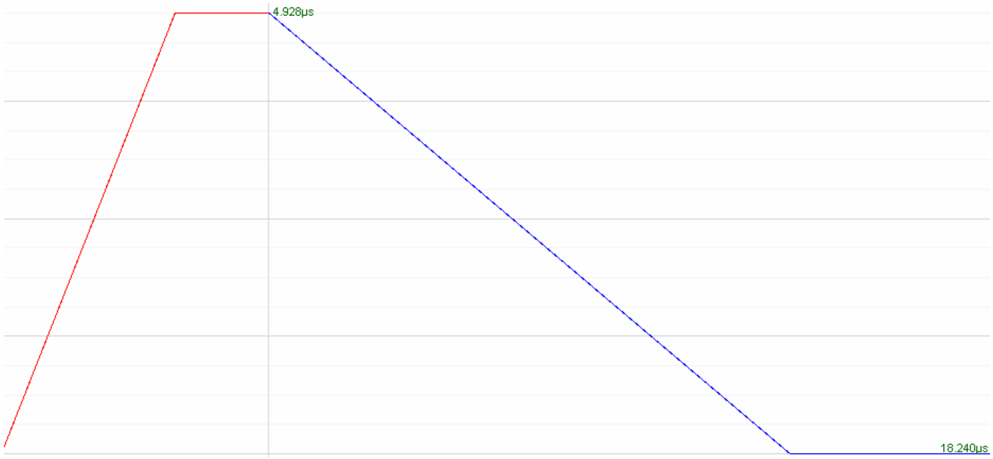


Figure AII3. pH_indicator *setting configuration jetting waveform*

II.1.4. Cleaning cycle: spot_purge0.1sec_spit

- Frequency: every 20 printing bands or 60 seconds
- Spit: 500 ms, 1.5 khz
- Purge: 1.0 ms
- Blot: 2.0 ms

II.2. EPSON settings

- Printing mode: 封筒
- Resolution: きれい

III. *Simple symmetry 6* (made with EPSON and Dimatix) patterns using non particle pH based sensor reagent. Colour and image data

III.1. Colour data

pH 5	Before test			7 min after test			25 min after test		
Pattern	L*	a*	b*	L*	a*	b*	L*	a*	b*
92	71.324	16.668	13.520	61.523	20.770	13.984	70.028	16.848	14.879
93	73.964	16.184	11.871	65.315	19.656	13.883	72.808	16.824	15.008
94	71.916	15.035	10.480	64.218	18.797	12.855	71.983	16.203	14.219
95	71.478	15.988	11.230	62.670	20.094	13.371	71.258	16.246	13.207

pH 6	Before test			7 min after test			20 min after test		
Pattern	L*	a*	b*	L*	a*	b*	L*	a*	b*
96	72.213	15.820	9.949	64.132	21.930	19.453	70.738	15.707	16.031
97	71.492	16.500	10.848	63.520	22.887	19.520	70.599	16.273	14.293
98	73.123	14.945	10.953	64.083	21.250	17.914	72.239	13.668	13.422
99	73.117	15.637	9.168	65.386	20.262	17.164	71.855	15.879	14.816

pH 7	Before test			5 min after test			17 min after test		
Pattern	L*	a*	b*	L*	a*	b*	L*	a*	b*
100	73.187	16.492	12.891				70.452	11.152	28.414
101	72.445	16.445	13.664				65.521	19.992	29.250
102	74.035	16.148	11.785				72.569	12.332	28.801
103	78.112	14.449	11.512	88.396	-0.816	22.488	88.716	-0.023	20.426

pH 8	Before test			5 min after test			10 min after test		
Pattern	L*	a*	b*	L*	a*	b*	L*	a*	b*
103	70.832	15.684	12.258	71.354	3.020	24.984	73.327	2.957	27.141
104	70.329	15.258	10.879	70.092	-0.434	22.527	71.505	1.945	24.348
105	70.350	15.438	12.324	69.102	0.473	23.492			
106	69.714	15.148	10.754				67.001	4.516	29.746

pH 8	15 min after test			20min		
Pattern	L*	a*	b*	L*	a*	b*
103				73.291	4.312	28.066
104				73.681	4.191	26.301
105	70.642	4.457	26.375			
106						

pH 9	Before test			5 min after test			14 min after test		
Pattern	L*	a*	b*	L*	a*	b*	L*	a*	b*
107	74.461	15.582	13.270	67.700	-6.918	4.434	66.815	-4.211	-2.121
108	74.618	15.555	13.195	57.029	-6.332	4.859	56.745	-4.664	-2.031
109	72.824	17.176	13.117	56.997	-5.730	4.367	58.624	-4.688	-1.691
110	74.578	15.492	13.316	58.767	-6.383	4.938	56.858	-4.789	-3.781

pH 9	23 min after test		
Pattern	L*	a*	b*
107	65.982	-1.996	-5.785
108	58.820	-2.539	-6.605
109			
110			

Table AIII1. Colour data of the Simple symmetry 6 patterns tested with pH buffers

III.2. Image data

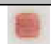
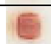
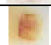
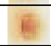
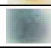
Pattern	pH buffer used	Before test	After test		After test	
		Image	Time (min)	Image	Time (min)	Image
93	5		7		25	
94						
97	6		7		20	
98						
100	7		17			
102						
104	8		5		20	
105					15	
109	9		5		14	
110						

Table AIII2. Image data of the Simple symmetry 6 patterns tested with pH buffers

Some images of the patterns are shown, too. As it can be observed, some of them were slightly affected by coffee ring effect, although this was not a serious problem.

Figure AIII1. Pattern 100 (pH 7), 17 minutes after the test

Figure AIII2. Pattern 101 (pH 7), 17 minutes after the test

Figure AIII3. Pattern 96 (pH 6), 20 minutes after the test

Figure AIII4. Pattern 106 (pH 8), 10 minutes after the test

Figure AIII5. Pattern 108 (pH 9), 23 minutes after the test

Figure AIII6. Pattern 110 (pH 9), 10 minutes after the test

IV. *Simple symmetry 6* (made only with EPSON) patterns using non particle pH based sensor reagent. Colour and image data

IV.1. Colour data

pH 5	Before test			14 min after test			20 min after test		
Pattern	L*	a*	b*	L*	a*	b*	L*	a*	b*
129	77.263	15.742	12.688	68.659	17.605	16.195	70.747	16.594	16.695
130	75.860	17.098	13.828	71.662	18.930	19.402	73.983	16.539	16.664
131	77.292	14.730	12.562	72.374	18.551	18.949	74.369	16.273	18.793
132	77.694	16.379	16.121	74.786	18.227	18.242	75.798	15.523	18.066

pH 5	23 min after test		
Pattern	L*	a*	b*
129	71.458	15.094	15.621
130	75.602	15.430	14.453
131	75.882	14.910	16.246
132	76.703	14.176	15.945

pH 6	Before test			13 min after test			23 min after test		
Pattern	L*	a*	b*	L*	a*	b*	L*	a*	b*
113	77.101	14.383	10.656	76.976	14.910	19.691	76.828	12.867	14.758
114	77.635	14.543	10.629	77.916	13.945	21.977	77.508	11.961	18.922
115	76.655	14.398	10.887	76.036	14.949	21.469	74.495	14.668	18.234
116	77.867	14.742	9.793	77.824	13.664	20.957	76.532	12.227	16.941

pH 7	Before test			10 min after test			16 min after test		
Pattern	L*	a*	b*	L*	a*	b*	L*	a*	b*
117	76.544	14.938	11.941	78.906	7.582	25.652	80.490	7.387	21.031
118	76.791	15.004	12.723	81.032	5.008	26.832	82.036	5.176	22.703
119	77.642	14.488	12.672	79.791	5.410	27.227	82.040	4.594	21.492
120	78.665	13.613	12.004	81.492	3.973	24.215	82.196	4.055	22.016
	Before test			10 min after test			15 min after test		
133	74.438	15.531	15.848	81.556	6.309	29.762	78.676	7.281	27.527

pH 7	22 min after test		
Pattern	L*	a*	b*
117	80.378	7.812	18.949
118	82.681	5.090	19.391
119	81.993	5.281	19.438
120	82.904	4.504	19.242
	20 min after test		
133	79.355	7.102	24.66

pH 8	Before test			11 min after test			16 min after test		
Pattern	L*	a*	b*	L*	a*	b*	L*	a*	b*
121	79.237	12.816	11.859	84.149	-1.617	24.250	85.858	-0.273	20.695
122	76.880	14.609	12.652	81.694	1.902	25.527	83.084	2.074	23.445
123	76.159	14.500	12.688	80.609	-2.238	26.672	82.960	-1.121	25.984
124	76.600	15.422	12.277	81.433	-2.871	26.555	84.215	-0.910	24.438
	Before test			10 min after test			15 min after test		
134	74.937	15.992	15.418	89.868	-3.223	13.754	87.597	-2.578	15.039
135	76.242	15.754	18.145	88.862	-3.297	21.203	86.011	-3.340	22.941

pH 8	23 min after test		
Pattern	L*	a*	b*
121	85.890	-0.098	20.059
122	82.835	1.785	23.074
123	84.228	-0.184	22.395
124	85.629	-0.059	21.262
	20 min after test		
134	87.700	-2.402	14.789
135	86.220	-2.086	22.160

pH 9	Before test			9 min after test			17 min after test		
Pattern	L*	a*	b*	L*	a*	b*	L*	a*	b*
125	76.771	14.367	12.461	73.883	-7.449	0.746	79.451	-4.691	-0.242
126	76.652	14.641	12.660	70.494	-7.320	0.051	75.404	-4.285	-1.270
	Before test			7 min after test			13 min after test		
127	74.861	15.852	15.805	77.807	-6.363	2.293	81.416	-5.051	1.613
128	73.892	16.363	16.332	70.582	-7.691	1.242	78.843	-4.656	0.848
136	76.144	16.297	18.031	76.184	-7.047	1.188	81.079	-5.105	0.852

pH 9	24 min after test		
Pattern	L*	a*	b*
125	78.744	-4.723	-0.949
126	75.053	-4.691	-2.348
	20 min after test		
127			
128	77.957	-4.426	-0.32
136			

Table AIV1. Colour data of the Simple symmetry 6 patterns tested with pH buffers using only EPSON printer

IV.2. Image data

Pattern	pH buffer used	Before test	After test		After test		After test	
		Image	Time (min)	Image	Time (min)	Image	Time (min)	Image
130	5		14		20		23	
131								
115	6		13		23			
116								
117	7		10		16		22	
118								
122	8		11		16		23	
123								
125	9		9		17		24	
126								

Table AIV2. Image data of the Simple symmetry 6 patterns tested with pH buffers

Some images of the patterns are shown, too. As it can be observed, some of them were strongly affected by coffee ring effect.

Figure AIV1. Pattern 130 (pH 5), 20 minutes after the test

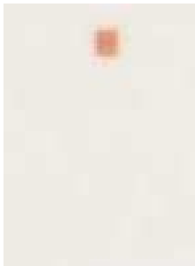


Figure AIV2. Pattern 131 (pH 5), 20 minutes after the test

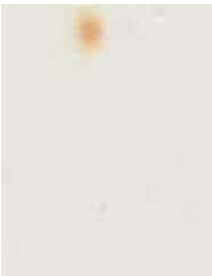


Figure AIV3. Pattern 117 (pH 7), 16 minutes after the test

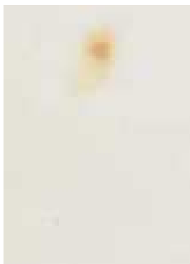


Figure AIV4. Pattern 118 (pH 7), 16 minutes after the test

Figure AIV5. Pattern 125 (pH 9), 17 minutes after the test

Figure AIV6. Pattern 126 (pH 9), 17 minutes after the test