

# AUTOMATED PNEUMATIC VALVE ACTUATION TO PERFORM MICROFLUIDIC PROTOCOLS

## INTRODUCTION

**Microfluidic technology** has been utilized in **multiple research** and **industrial fields**, providing advantages in fluid **handling capabilities**, **scalability**, **robustness**, and **automation**. In the past 10 years, effort has been devoted to the **development of microfluidic platforms** capable of performing **several assays** using **programmable fluidic operations** within an array of microvalves. Similar to logic circuits where multiple computing routines are executed on a single device, programmable microfluidic platforms have been implemented<sup>3</sup>. This allows one to perform **fluidic operations** such as mixing, sampling, washing, and reacting automatically **on a microfluidic chip** by modifying the sequence/order of fluidic operations with software. This is useful in **biological and chemical applications**, such as **quantitative metabolic biomarker and genetic analysis**<sup>4,5</sup>, **protein-based biomarker detection**<sup>6</sup>, or **small molecule chemical and environmental analysis**<sup>7</sup>. These platforms usually consist of a 2D-array of microvalves that permit flow regulation, on/off switching and sealing of liquids, gases or vacuums<sup>8</sup>. Several microvalves have been developed using pneumatic, electrokinetic and electrochemical actuators. Among these mechanisms, **pneumatic actuation** is the **most reliable** due to the **simplicity of fabrication**, **ease-of-use**, **scalability**, and **high degree of accuracy, precision, and reliability**. Pneumatically actuated microvalves utilize the deflection of an elastomer (typically PDMS) membrane to control fluid flow<sup>1</sup>. Today, most platforms in the academic field are customized: solenoid valves are connected to a specially designed optocoupler, all contained in a custom casing. This is monitored using a microcontroller connected to PC, and a customized software is implemented for controlling the valves and sequence/order of fluidic operations. An additional software is required for controlling pressure (figure 1 on next page). As a result, setting up the infrastructure to control such platforms is often **time-consuming**, and requires **specific engineering expertise** and extensive research facilities. As a consequence, a **major barrier** to entry for using microfluidic devices with integrated valves can be implementing control software and electronics<sup>9</sup>.

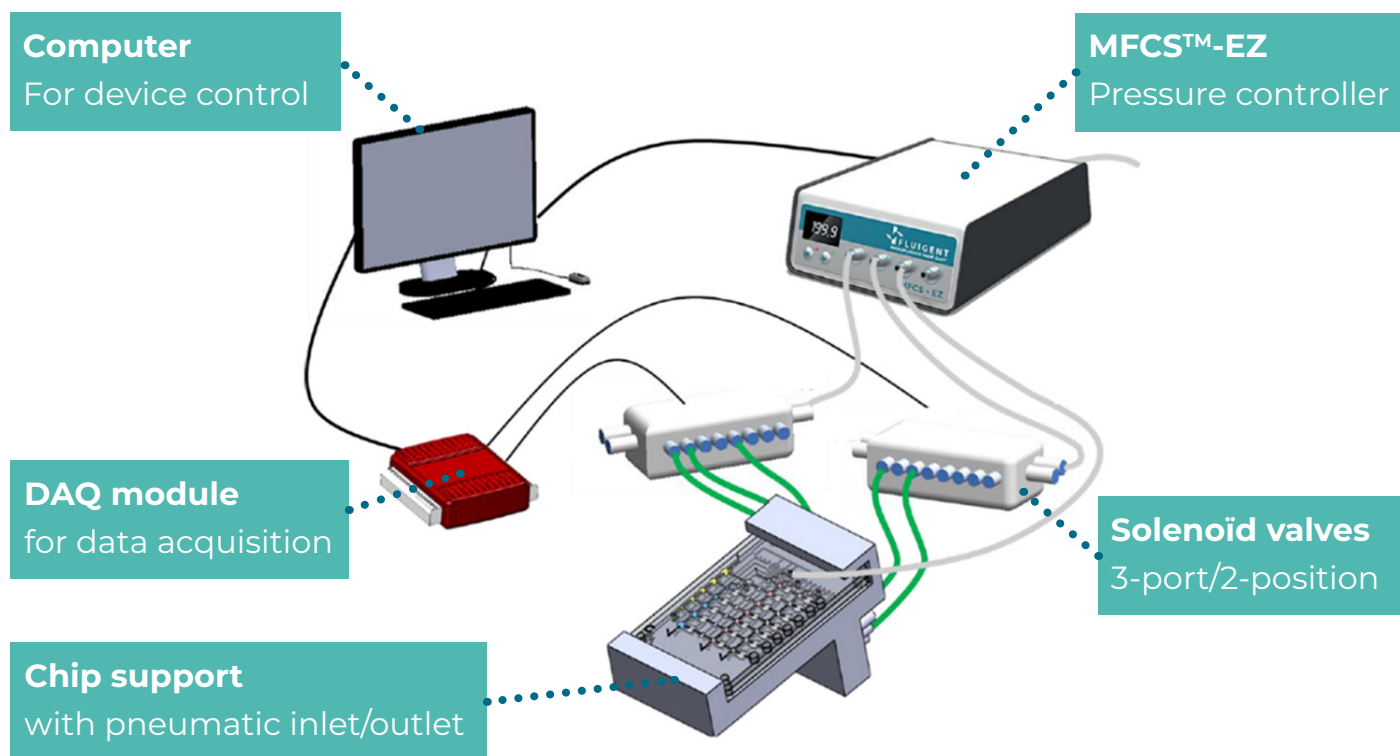


Figure 1: Schematic of a typical microfluidic platform with integrated valves<sup>10</sup>

The **LineUp™ P-SWITCH** is designed to address these concerns: When combined with pressure controllers, it is a **ready-to-use microfluidic electrovalve controller** (positive and negative pressures) that can connect to **any microfluidic system with pneumatic channels**. Using **Fluigent MAT software**, it is possible to implement **complex sequencing of fluidic operations** with accurate pressure steps, without requiring additional materials or software. Elementary fluidic functions such as **fluid transfer, volume calibration, mixing, aliquoting** and **linear dilutions** can be **parallelized, automatically controlling** complex operations.

We demonstrate the use of the **LineUp™ system** with a **P-SWITCH** and using **MAT software** for the sequential injection of fluids within a microfluidic chip to perform **pumping, mixing, sampling, and cleaning**.

## MATERIALS

### MICROFLUIDIC PRESSURE BASED CONTROL



#### LineUp™ P-SWITCH

This module contains 8 **3-port/2-position solenoid valves**. It allows one to **switch between 8 pressure outlets** and **2 different supplied pressures** (P1 and P2). Those pressures are common to all the valves and can be controlled within the **range of - 800 mbar to + 2000 mbar**. It can be used to actuate **quake** or other **pneumatic valves**.



#### LineUp™ Flow EZ

The most advanced flow controller for **pressure-based fluid control**. The **Flow EZ™** is available in a variety of pressure ranges from **-800 to +7 000 mbar**. It can be combined with a **FLOW UNIT** to control pressure or flow rate. Two Flow EZ™ 1 000 mbar and one Flow EZ™ -800 mbar are used here.



#### LineUp™ LINK

The **LINK** provides connection of LineUp™ series modules to a **PC for software control**. Fluigent software solutions allow one to **control and monitor** the devices in real time as well as **record data (All-in-One)**, or to **automate pressure/flow-rate steps** and **microfluidic valve actuation (MAT)**.

## MICROFLUIDIC CHIP

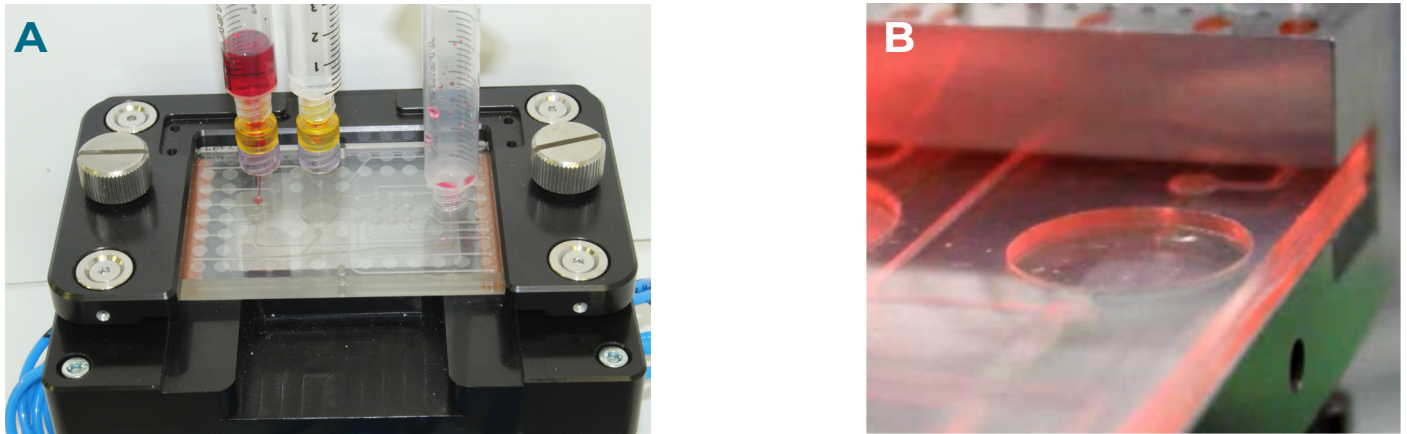


Figure 2: (A) Chip holder + Flowstretch microfluidic chip with valve chambers to actuate liquids directly from syringes, (B) Focus on a chamber

The **microfluidic system** used was designed by the **CEA-Leti**. The device is a hybrid card consisting of **three polymer cards** and a **stretchable membrane**, illustrated in figure 3.A below. The first card contains reagents storage and network channels to connect the **fluidic chambers** (1). The second card contains **pneumatic valves and chambers** at the top layer and pneumatic network at the bottom layer (2). The third card allows for **the pneumatic channels** to close (3). The stretchable membrane is positioned between the fluidic card (1) and the pneumatic card (2). The elastic membrane is made of Ecoflex00 50, a bi-component silicone material that withstands larger deformations than PDMS, allowing to totally empty and fill the chambers at low pressures (few hundreds of mbar), which simplifies the pneumatic instrumentation required to actuate the chip.

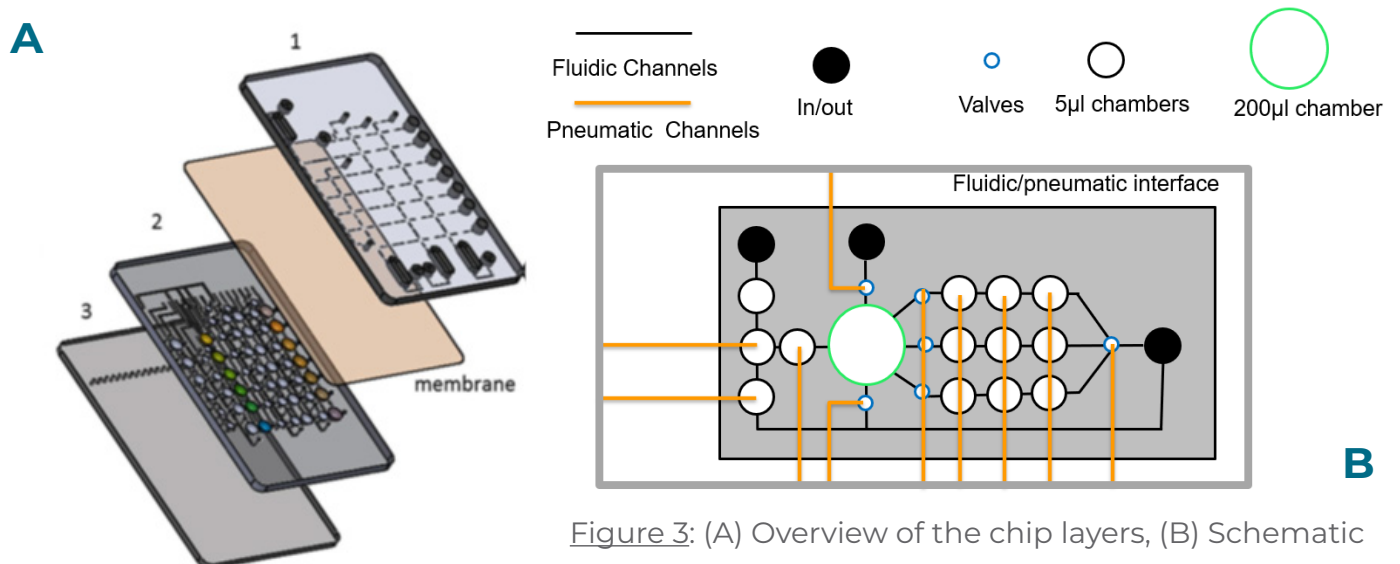


Figure 3: (A) Overview of the chip layers, (B) Schematic representation of the chip chambers and connections.

# APPLICATION NOTE

The chambers and valves shift between two states. The fluidic system is **closed** when a **positive pressure is applied** to the chamber or the valve and **open** when **negative pressure is applied**. The fluidic network channel consists in **13 chambers of 5  $\mu\text{L}$**  and **1 chamber of 200  $\mu\text{L}$** , allowing for pumping, mixing and sampling operations.

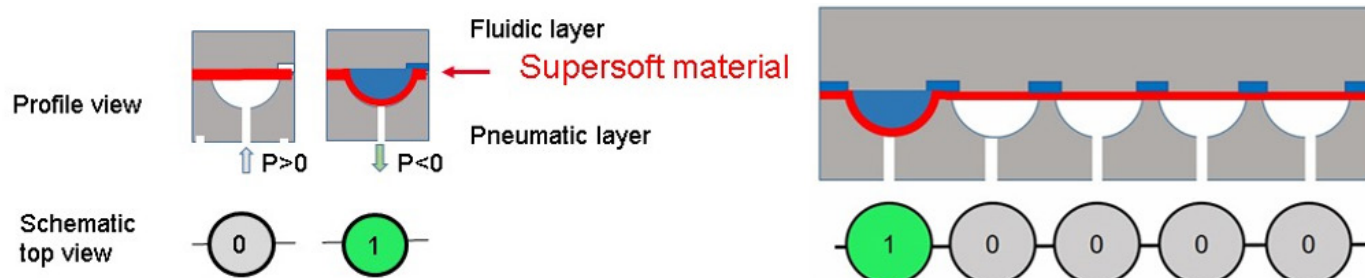


Figure 4: Working principle of the chip quake valves

## SOFTWARE CONTROL AND AUTOMATION



The **Fluigent Microfluidics Automation Tool (MAT)** is a software package for developing and running time based microfluidic protocols using Fluigent instruments. It allows one to easily create **protocols for automated experiments**, including a **wide range of operations and loops** as well as TTL input/output.

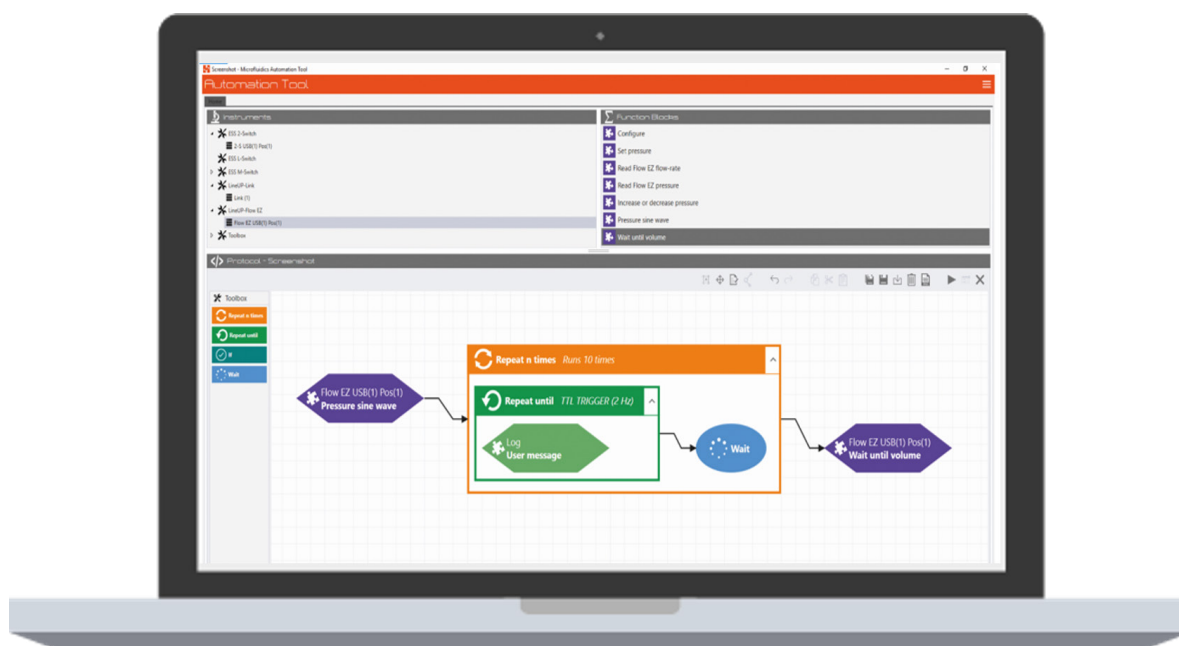
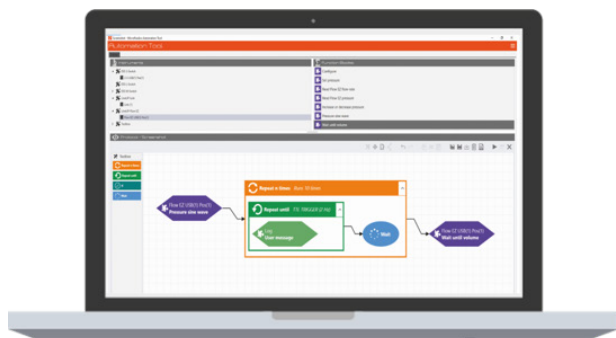


Figure 5: Overview of Fluigent Microfluidics Automation Tool interface (protocol shown unlinked to the current application note)

## METHODS & RESULTS

### SYSTEM SET-UP

**Computer with MAT software**  
Automation tool for the protocols

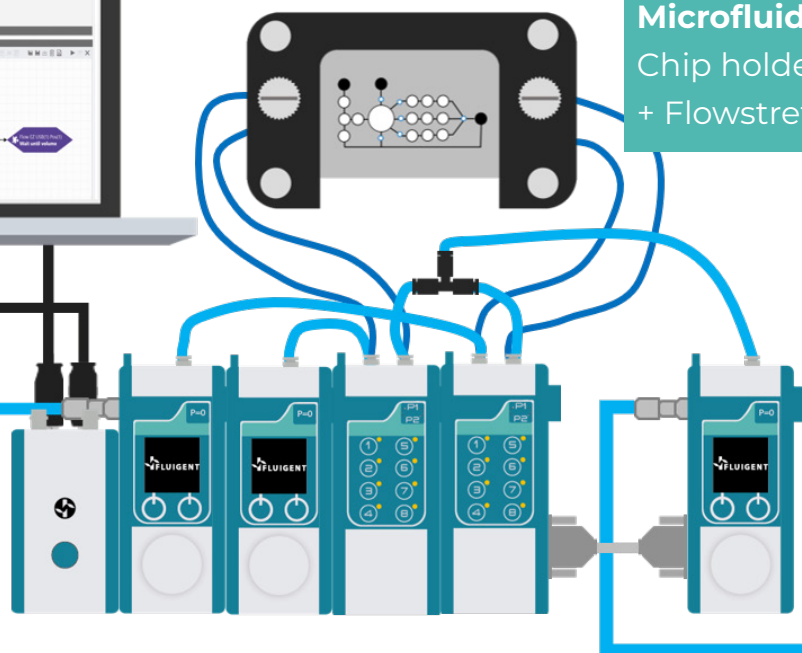


**Power supply**

**Pressure supply**

**LineUp controllers chain**

LINK, 2 Flow EZ 1 bar,  
2 P-SWITCH, 1 Flow EZ -800  
mbar



**Caption**

— Pneumatic tubing 4mm OD

— Pneumatic tubing 3mm OD

**Microfluidic system**  
Chip holder with inlets  
+ Flowstretch chip

**Vacuum supply**

Figure 6: Complete system set-up schematic with devices, supplies and pneumatic tubing connections

The system used is shown in figure 6 above. An external pressure source is connected to the **LineUp™** system consisting in a **LINK** module, two **P-SWITCH™**, two **Flow EZ™ +1000 mbar** and one **Flow EZ™ -800 mbar**. Each Flow EZ™ supplied with positive pressure is connected to one distinct P-SWITCH™ while the Flow EZ™ with negative input is connected to both P-SWITCH™. This way each quake valve controller (P-SWITCH) is able to **provide positive pressure** or **use a vacuum** on the chip chambers. The **8 outlets lines** from one P-SWITCH™ are **connected to the pneumatic chambers** of the microfluidic system, while the outlet tubing from the second P-SWITCH™ is connected to the pneumatic valves. The **chambers are pressurized at +100 mbar**, the **valves are pressurized at +450 mbar**, and the **negative pressure applied is - 100 mbar** on the whole pneumatic system. **Syringes** containing red dye and distilled water are **directly connected to the two inlets** of the microfluidic chip, and a syringe is connected to the **outlet to collect waste**.

## AUTOMATION OF SEQUENCED FLUIDIC OPERATIONS

In order to **automate the fluidic operation steps**, the Fluigent **Microfluidic Automation Tool** (MAT) software is used. A new sequence block is added to the protocol section. To help building the protocol, a **picture of the microfluidic chip is uploaded**, and each chamber and valves are annotated. Here, **A and B represent the two P-SWITCH** and the **numbers represent the valves** buttons of each P-SWITCH (figure 7).

Each **P-SWITCH** module actuates **8 valves on the microfluidic chip**. On the MAT software, those **valves are indicated with a letter (A or B)** for the module, and a **number from 1 to 8 for the corresponding outlet**.

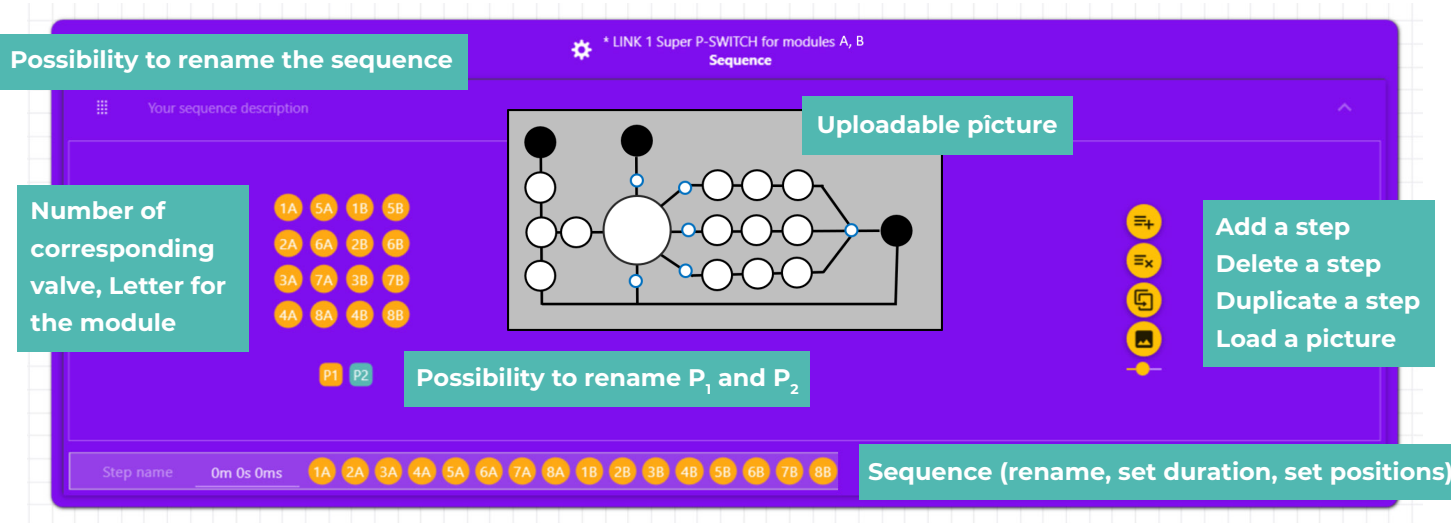
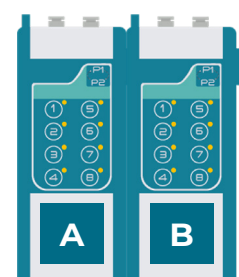


Figure 7: MAT function bloc to automate pressure steps using P-SWITCH

The step sequences are next edited by modifying the valve position. When the **valves are displayed in green**, it corresponds to a **positive pressure** (closed fluidic system: the liquid cannot flow in the chamber/valve), when the **valves are displayed in orange**, it corresponds to a **negative pressure** (open fluidic system: the liquid is allowed to flow in the chamber/valve). “Pumping”, “mixing”, “sampling,” and “cleaning” steps are edited. The **steps are implemented sequentially**, with **specific duration time**. Note that it is **also possible to do it manually** using the **LineUp™ local control feature**.

## PUMPING

In order to ensure **precise fluid delivery**, a pumping step is often performed within a microfluidic chip, provide **accurate liquid control** and compensate for swept or dead volumes. The liquid is introduced through the microfluidic chip for filling these volumes. To illustrate this process, a red dye is injected, and flows sequentially through the chambers 6B, 5B, and 4B, before exiting the chip through the outlet. **This is performed by applying vacuum** to the selected fluidic chambers, as shown in figure 8 below.

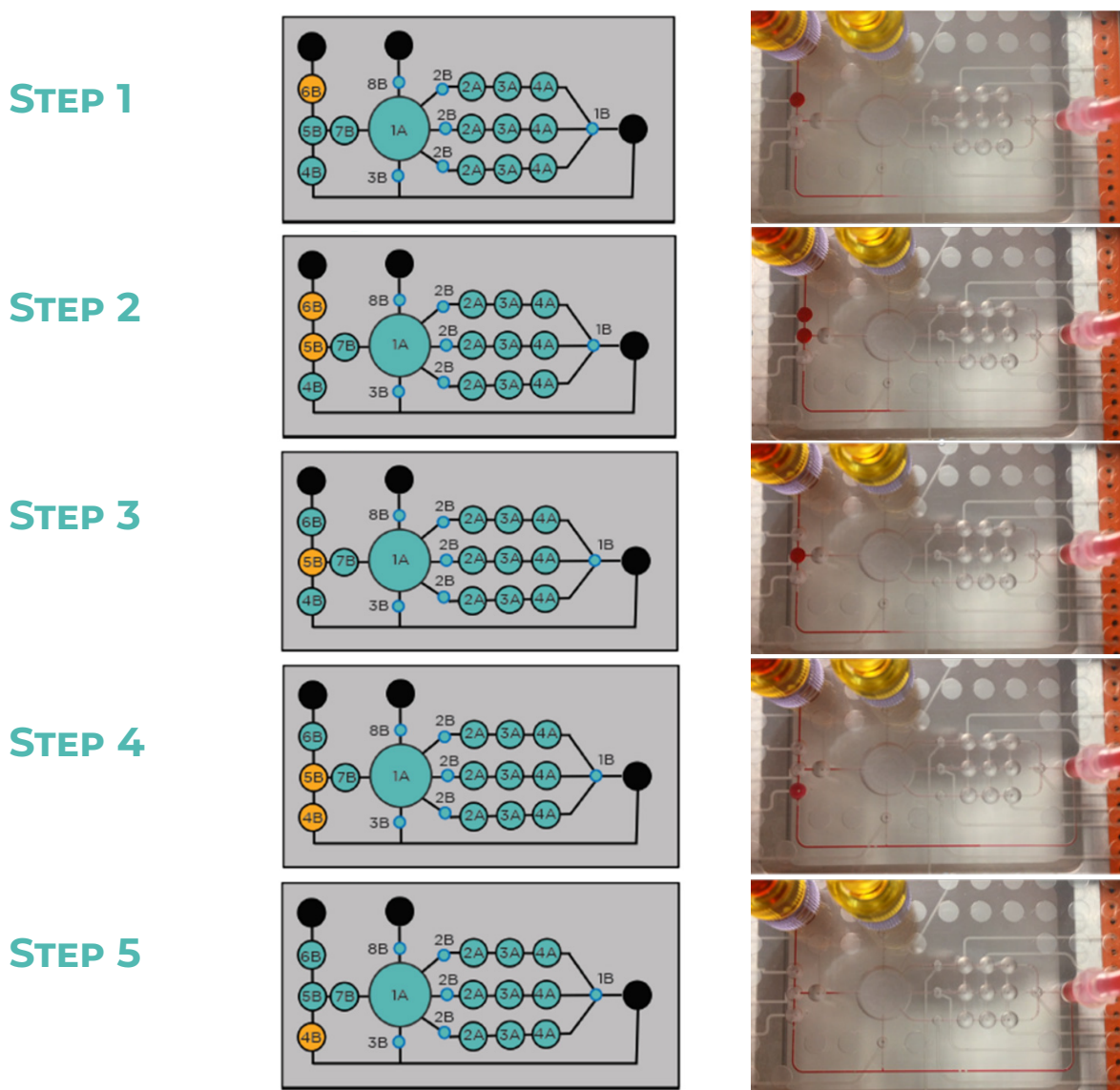
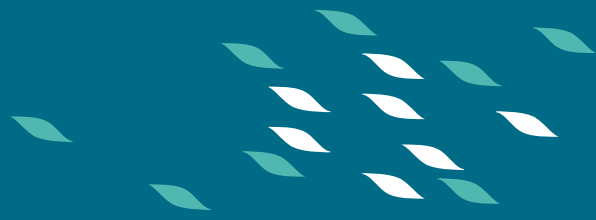


Figure 8: Valves steps to perform the automated pumping protocol

Figure 8 shows the **pumping within the microfluidic chip**. We can observe that **fluid is precisely injected** on the targeted chambers for each steps, and that it fully exits the chip at the end of the sequence. A 6<sup>th</sup> step is added to close all valves. The protocol is performed in **less than 5 seconds**.

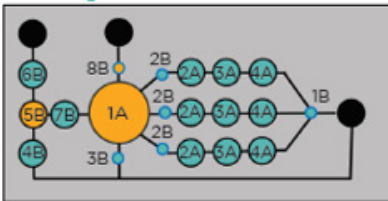


## MIXING (CALIBRATION)

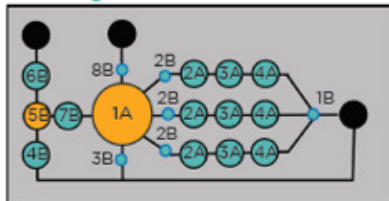
Mixing, is often required for **sample dilution**, reagent **homogenization**, and chemical or biological reactions. **Mixing can be challenging under microfluidic conditions** due to **laminar flow**. Turbulent flow (improving mixing) is prevented. By using pneumatic actuation with a membrane deflection, fluid motion between the chambers turns out to be very fast<sup>1</sup>. This can be used to **create fluidic recirculation between two solutions** within a chamber, and **enhance mixing**. To improve the mixing performance, liquids can also move back and forth by actuating the chambers again. Here, an automated protocol was developed to demonstrate fluid mixing between two solutions using pneumatic actuation. A red dye solution of 5  $\mu\text{L}$  is moved to the 5B chamber of the chip, and 200  $\mu\text{L}$  of distilled water is moved to 1A chamber. Next, the red dye is moved to the 7B chamber, and finally moves to 1A for mixing. **Liquid is moved back and forth** by actuating the chamber 7B 3 times to **ensure complete mixing**.

### Calibration of 200 $\mu\text{L}$

#### Step 1

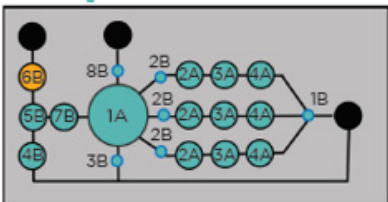


#### Step 2

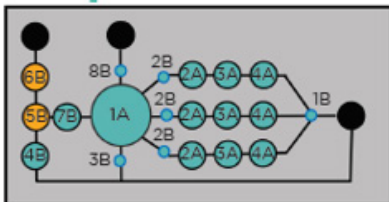


### Calibration of 5 $\mu\text{L}$

#### Step 1



#### Step 2



#### Step 3

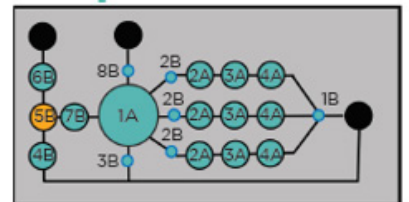


Figure 9: Valves steps to perform calibration of 5 $\mu\text{L}$  and 200 $\mu\text{L}$  volumes

## MIXING

Figure 10 shows the solutions positions after calibration. The red dye solution is correctly moved to 5B position, and distilled water is moved to 1A.

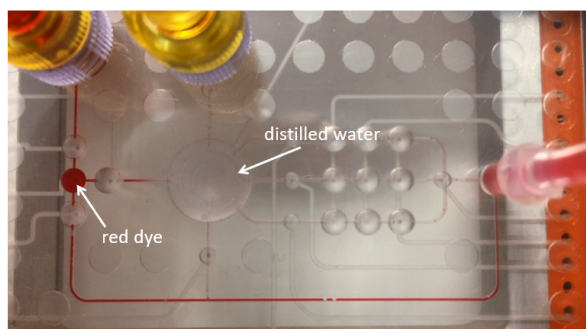
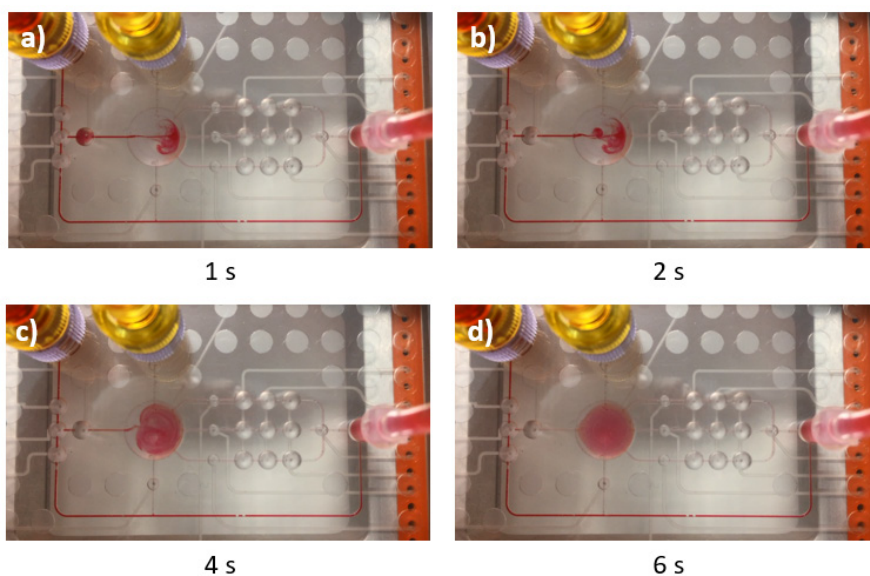
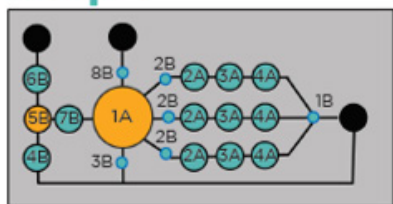


Figure 10: Microfluidic chip state after calibration protocol, ready to mix

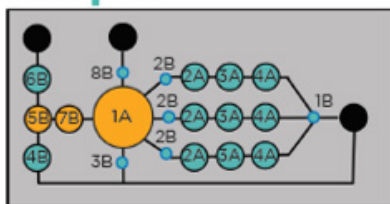
Figures 11 (A) and (B) show the injection of the red dye solution to the 200  $\mu$ L chamber containing distilled water (1A position), and figures 11 (C) and (D) show the **consecutive re-injections** for improved mixing. **Fluidic recirculation can be observed** and the solution seems **homogeneous** after three fluid transfers, in **less than 6 s**.



### Step 1



### Step 2



### Step 3

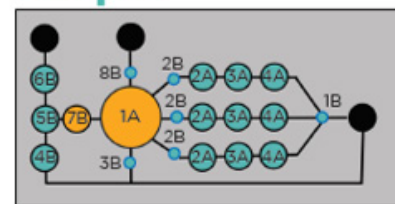


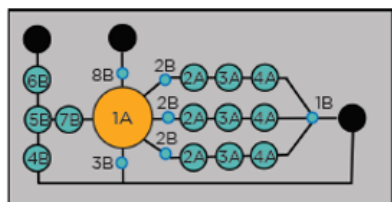
Figure 11: Valves steps to perform mixing protocol

## SAMPLING

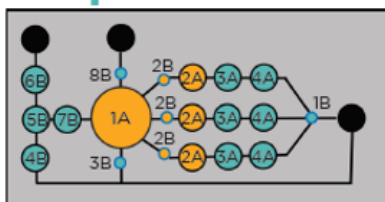
The process of sampling is also **typically performed in fully integrated micro total analysis system** or **Lab-on-a-Chip devices**. To demonstrate this process using our system, the solution previously mixed in 1A is sampled into 3 chambers, each connected to 2 consecutive chambers. The complete protocol is described in figure 12. Briefly, the 2B and 2A valves are open, allowing to move to solution into the 2A chambers, and subsequently to the consecutive chambers (3A and 4A), before exiting the chip through the outlet.

### Step 0

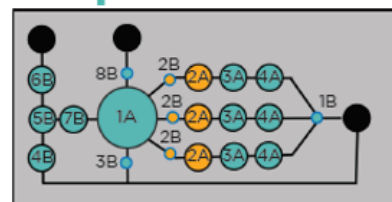
(Initial configuration after mixing)



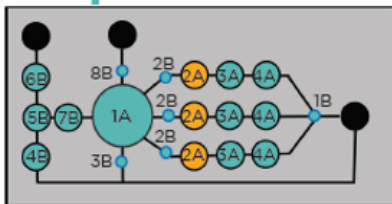
### Step 1



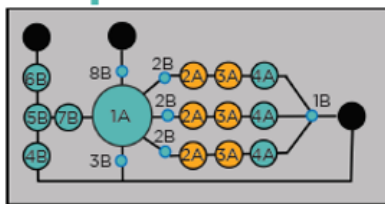
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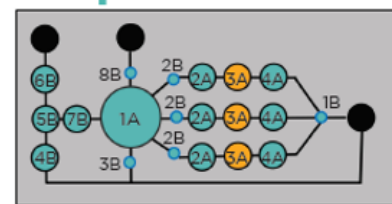
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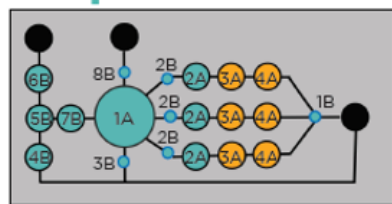
### Step 4



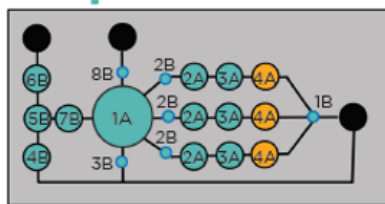
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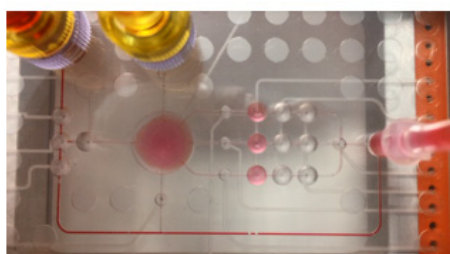
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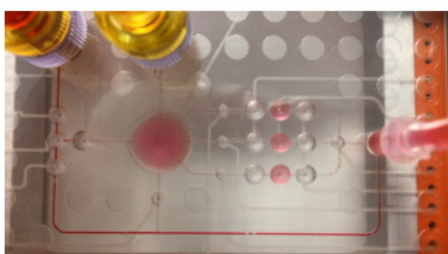
### Step 7



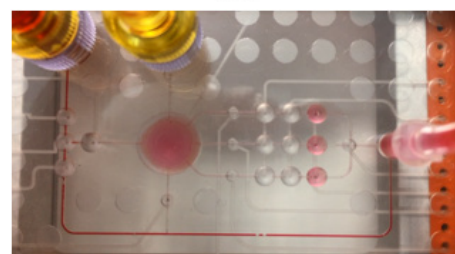
1 s



2 s



3 s



4 s

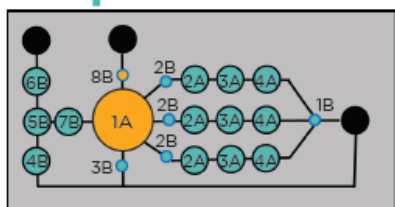
Figure 12: Valves steps to perform calibration of 5 $\mu$ L and 200 $\mu$ L volumes

Figure 12 shows the sampling process. The red solution is **equally dispensed into the three chambers** (2A) in less than 2 s. The solution is next moved to the consecutive chambers, as implemented on the protocol, and **no solution is remaining within the previous chambers** (2A and 3A). The sampling protocol functioned well, and took **less than 4 s**.

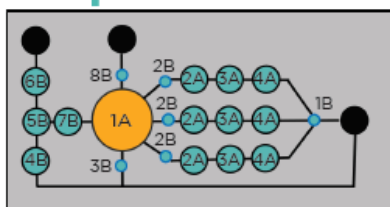
## CLEANING

At the end of a microfluidic protocol, **cleaning a reusable chip is needed** to ensure proper function for the next experiments. Here, an automated protocol is developed to perform a cleaning step. The complete protocol is described in figure 13. Briefly, distilled water is moved to the 200  $\mu\text{L}$  chamber. Next, the neighboring chambers (5B, 7B, 2B, 2A, 3A and 4A) are open, allowing for the liquid to flow into them. The **chambers are consecutively closed** and the **liquid is pushed through the outlet** of the microfluidic system, to the waste syringe.

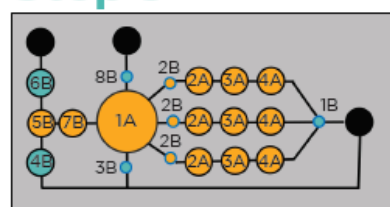
### Step 1



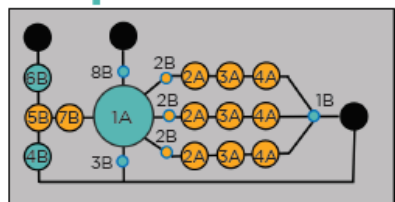
### Step 2



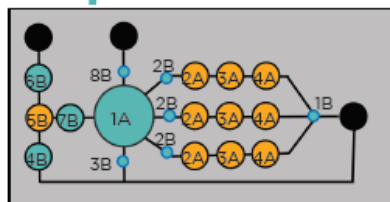
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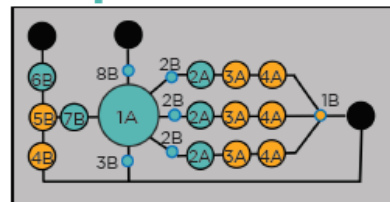
### Step 4



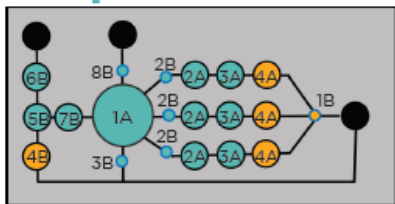
### Step 5



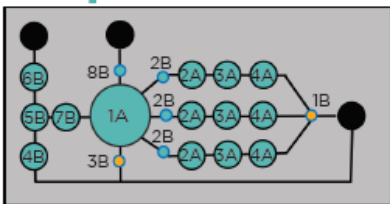
### Step 6



### Step 7



### Step 8



### Step 9

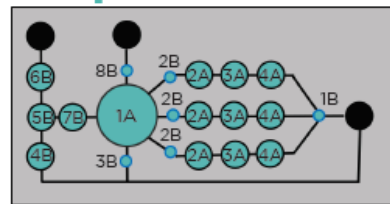


Figure 13: Valves steps to perform cleaning protocol

Figure 14 shows the cleaning process. This step is implemented right after the sampling protocol. We observe in figure 14 (A) that distilled water is injected within the 200  $\mu$ L chamber, removing the red solution from the chamber. Figure 14 (B) shows the microfluidic chip at the last step of the cleaning process. We observe that there is no red dye remaining in the microfluidic chambers, confirming the functioning of the cleaning step.

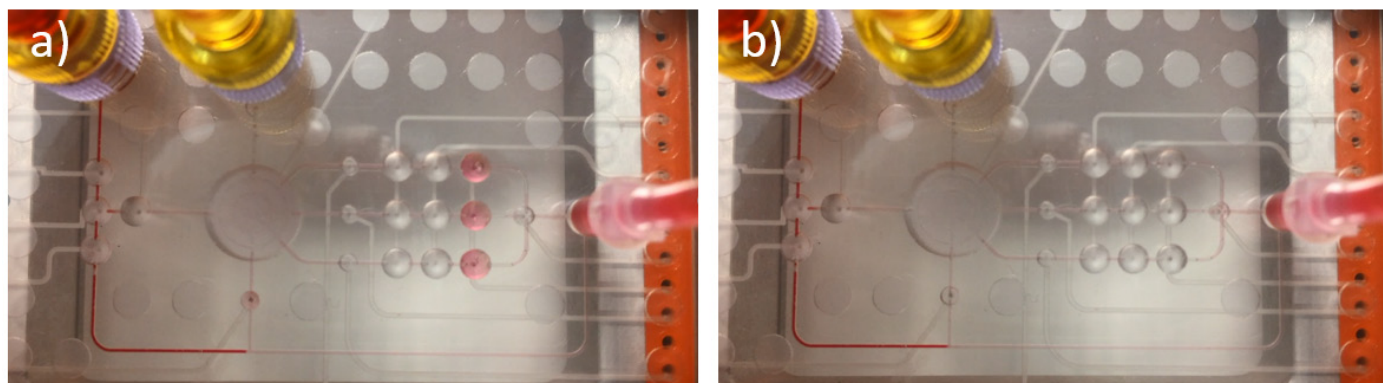
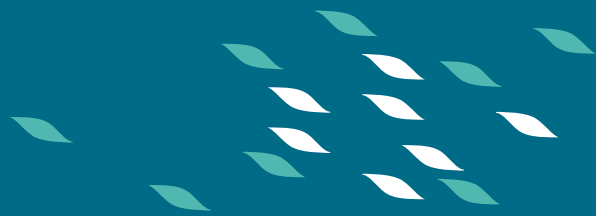


Figure 14: Microfluidic chip state during cleaning protocol, after sampling

## CONCLUSION

We have demonstrated the use of the **LineUp™ P-SWITCH** and Fluigent **MAT software** for implementing **sequential fluid operations**, including **pumping, mixing, sampling** and **cleaning**, with a custom microfluidic chip. Using the P-SWITCH brings many advantages such as **quick changing of liquids, no liquid contact** with the instrument, **less cross contamination** (compared to fluidic valves). Moreover, the **system is expandable** as several P-SWITCHes can be stacked. This microfluidic system is useful in many applications such as:

- Quake/Mathies valve control
- Fast medium switching
- Sequential sample injection
- On-demand droplets
- Medium perfusion switch for cell biology
- Microfluidics in flow chemistry, cell culture, ...



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## LIST OF FIGURES

<a href="#">Figure 1</a> : Schematic representic a typical microfluidic platform with integrated valves <sup>10</sup>	1
<a href="#">Figure 2</a> : (A) Chip holder + Flowstretch microfluidic chip with pneumatic valve chambers to actuate liquids directly from syringes, (B) Focus on a chamber	3
<a href="#">Figure 3</a> : (A) Overview of the chip layers, (B) Schematic representation of the chip chambers and connections.	3
<a href="#">Figure 4</a> : Working principle of the chip pneumatic valves	4
<a href="#">Figure 5</a> : Overview of Fluigent Microfluidics Automation Tool interface (protocol shown unlinked to the current application note)	4
<a href="#">Figure 6</a> : Complete system set-up schematic with devices, supplies and pneumatic tubing connections	5
<a href="#">Figure 7</a> : MAT function bloc to automate pressure steps using P-SWITCH	6
<a href="#">Figure 8</a> : Valves steps to perform the automated pumping protocol	7
<a href="#">Figure 9</a> : Valves steps to perform calibration of 5 $\mu$ L and 200 $\mu$ L volumes	8
<a href="#">Figure 10</a> : Microfluidic chip state after calibration protocol, ready to mix	9
<a href="#">Figure 11</a> : Valves steps to perform mixing protocol	9
<a href="#">Figure 12</a> : Valves steps to perform calibration of 5 $\mu$ L and 200 $\mu$ L volumes	10
<a href="#">Figure 13</a> : Valves steps to perform cleaning protocol	11
<a href="#">Figure 14</a> : Microfluidic chip state during cleaning protocol, after sampling	12