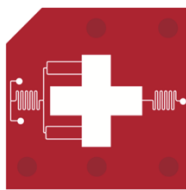




Sensus



SwisSense

Results Document

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Summary for the SensUs website

SwisSense is an interdisciplinary team composed of master students from microengineering, bioengineering, materials engineering and management faculties at Ecole Polytechnique Fédérale de Lausanne (EPFL, Switzerland). EPFL is one of the main centers of innovation hosting many state-of-the-art labs and supporting many spin-off start-ups, which have helped us throughout our journey.

After investigating different detection techniques, we opted for a fluorescence-based microfluidic approach based on the mechanically induced trapping of molecular interactions technology (MITOMI) developed by Prof. Sebastian Maerkl. The microfluidic chip is composed of a freestanding membrane that can be actuated by applying a differential pressure. When pressurized this membrane act as a “valve”, that expands and physically blocks liquids, enabling the functionalization in specific areas.

The NT- proBNP detection process takes place in a portable device which has great point-of-care application potential. The multiplexing capacity of this technique allows our device to detect other molecules simultaneously using the same whole blood sample and give a complete diagnostic on the patient’s cardiac health. Our advisors include experienced entrepreneurs, thought leaders in microfluidics and a cardiologist, that helped us to have a multidisciplinary approach to every problem and fit with the needs of both patients and doctors!

1. Biosensor System and Assay

1.1. Prototype

Our prototype consists of a microfluidic control system with pneumatic electrical valves and a pumping system, both controlled by an Arduino, a fluorescence USB microscope, the microfluidic chip acting as cartridge and a computer. The pressure in the chip is driven by the microfluidic control system and the fluorescence images are obtained with the USB microscope. The whole process is controlled and analysed with a homemade software.

A mechanical system enables an easily connection of the chip to the pumps and interaction with the user.

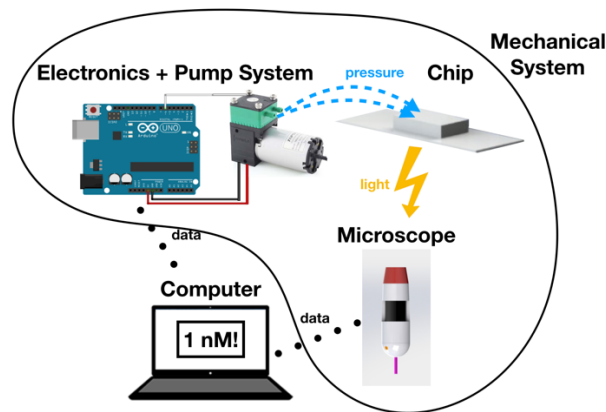


Figure 1: SwisSense biosensor system

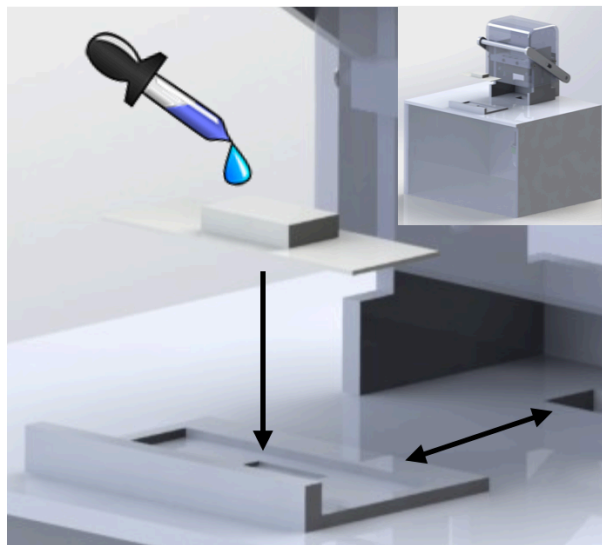


Figure 2: Plug & play chip into the system after loading of sample. The electronics are at the bottom part with an opening for the USB fluorescent microscope directly aiming at the backside of the chip.

1.2 Chip

We are using a PDMS microfluidic chip based on the MITOMI technology, combined with a USB fluorescent microscope. The protein NT-proBNP is captured using a sandwich immunoassay (see Figure 4). The chips are composed of an epoxy-coated glass slide, and two layers of PDMS channels (fabricated in clean room, see Appendix for more details) which consist of a flow and a control layer (Figure 3).

The flow is the layer at the bottom, where the sample will pass. It is pressure-driven by pumps and controlled by valves present in the control layer just above. Moreover, the control layer includes a button array, which when actuated by hydraulic or pneumatic pressure, will protect a circular area from the solute through physical contact on specified chambers. The cartridges used are functionalized in several steps (see Figure 4). The MITOMI technology allow us to functionalize only a specific region of the channels, under the buttons and to concentrate the antigen present in the sample.

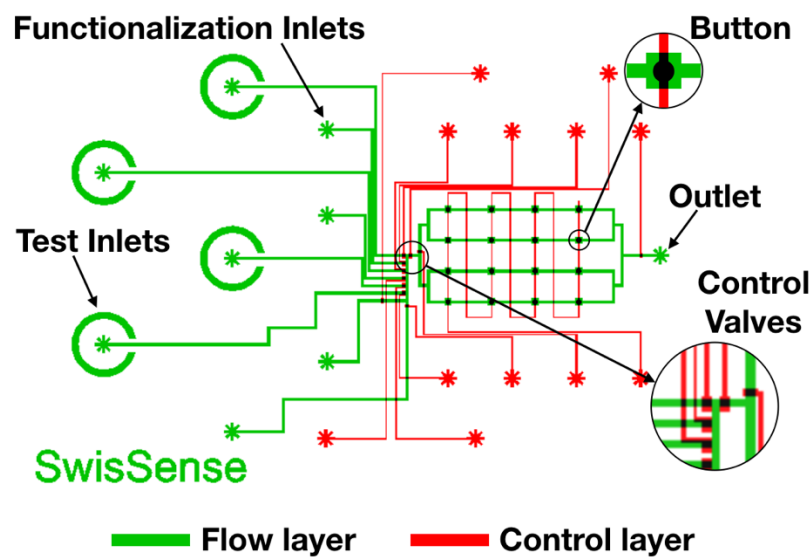


Figure 3: Microfluidic design: the device consists of two PDMS layers: flow (green) and control (red). When pressure is applied to the control layer, the valves and the MITOMI buttons are deformed downwards on the flow layer. The chip is an array of four rows by four columns for 16 unit cells. Each unit cell is composed of a chamber and one MITOMI button. There are two type of inlets: the Functionalization inlets, used for surface preparation and the Test Inlets, used to load the sample and the labelled antibodies. Each inlet is controlled by one corresponding valve.

1.3 Assay

The primary antibody used is anti-proBNP 15C4 (IgG2b, biotinylated). The secondary antibody is the 13G12 (IgG2a, non-biotinylated). The fluorescent antibody is a Goat anti-Mouse Alexa Fluor 488. It is IgG2a Cross-Adsorbed in order to specifically bind to the 13G12 only and limit the unspecific binding.

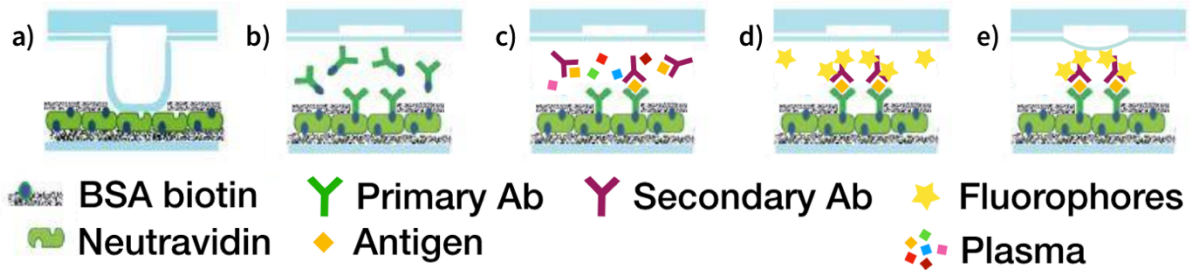


Figure 4: Schematic of one-unit cell and cross section of a button region. (a) Surface functionalization: BSA-biotin is flown through the chip bonded to epoxy-treated glass slide, followed by neutravidin. Next, the buttons are closed and BSA-biotin is flown again leaving free neutravidin molecules under the MITOMI buttons. (b) The primary biotinylated antibodies attach themselves to the available sites of neutravidin under the buttons. (c) The sample is premixed with the secondary antibodies and flown through the chip. The antigen-secondary antibody complex is captured by the surface immobilized antibodies. (d) Finally, the fluorescent-labelled tertiary antibody is flushed and binds specifically to the secondary antibody. (e) The sandwich-assay is trapped by the MITOMI button and quantified using a fluorescence USB microscope.

The fluorescence images obtained will then be processed and an intensity is calculated through signal processing.

This technique has several advantages as it can protect the surface of molecules and be used for the surface patterning. Also, it can prevent dissociation of the bound molecules by mechanically trapping them.

Such a system would allow multiplexing, allowing multiple tests at the same time as well as testing control samples to remove unwanted noise signal from the blood plasma.

2. Analytical Performance

2.1 Measurement data

Due to several problems related to the manufacture of the microfluidic chips, and the assembly of the electronic and mechanical part, we have not been able to make enough successful experiments in plasma and at low concentrations in order to obtain presentable data and graphs. However, we are actively working to ensure that this will be the case at the competition.

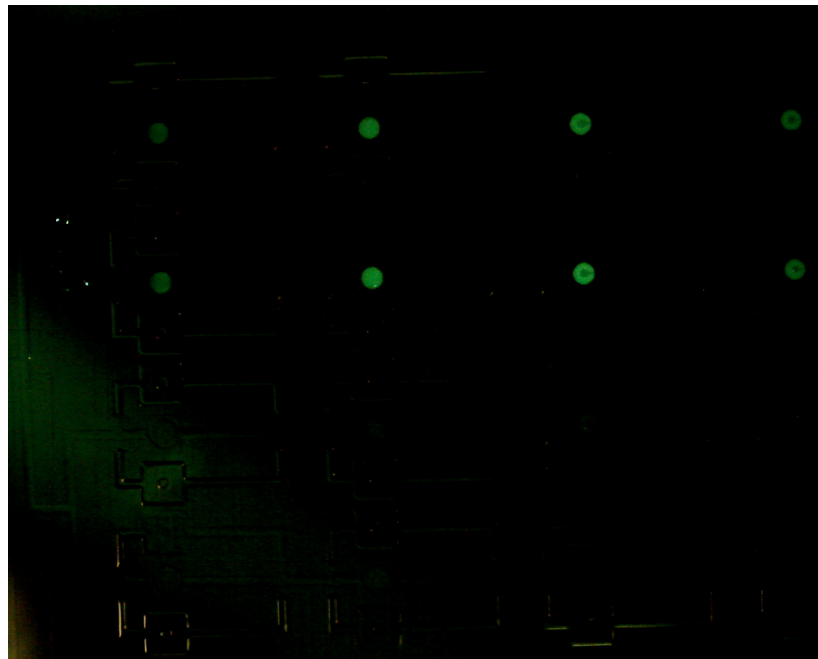


Figure 5: Image obtained in the lab at 10 ng/ml in PBS (Phosphate-buffered saline) solution.

Nevertheless, we present an image obtained with the microscope with the NT-pro BNP protein at 10 ng/ml. The circular zones, functionalized by the membranes emit fluorescence light. The intensity is then analysed to deduce the concentration. The lower buttons did not illuminate because a solution without antigen was introduced at the bottom and with antigen at the top, providing a control of the immunoassay.

2.2 Assay protocol

The biosensor is user-friendly and intuitive to use. Each test is done with a kit consisting of one cartridge and 3 reagents. The minimum required sample volume is 10 μ l. First, the user mixes the sample with the first reagent containing secondary antibodies. The mixture is then loaded on the pre-functionalized cartridge with a pipette. Simultaneously, the second reagent containing the

fluorophores and PBS (for washing steps) are loaded in different inlets. Finally, the cartridge is inserted in the reader. The rest of the process are fully automated and controlled by a software. Regarding the time-to-result since we couldn't perform rigorous testing due to lack of time and several issues, we cannot ensure specific numbers. Today tests done in the lab take approximately 30min after pre-functionalization (all the surface chemistry till the primary antibodies). However, we aim to detect the concentration after 8 min with the automated prototype which we develop thoroughly to be ready for the competition.

3. Novelty and Creativity

3.1 Already available

The mechanically induced trapping of molecular interactions concept (MITOMI) by using valves and membranes in PDMS was developed by Prof. Sebastian Maerkl [12]. The surface chemistry has been already used [11] and an example of setup using a USB microscope was as well explored. [1].

3.2 New developments

3.2.1 Technological aspects

While the technology to close the valves and the design of the buttons was existent, we designed a new microfluidic chip using the MITOMI principle that would be compatible with our read-out technique and mechanical system and optimize the assay protocol.

A great achievement of our team is to have built a fully integrated microfluidic pressure control system and readout in a portable way. In order to do so a monitoring module was created to synchronize the valve control and the USB microscope, with embedded post image processing analysis.

A plug & play mechanical system was completely designed and fabricated by our team to quickly and easily connect the chip to the pump system. While being simple, this system is also sufficiently robust to ensure the connection of the chip without any pressure the quick replacement of the chip (simple handling).

3.2.2 Approaches

Since our university hosts many innovative labs, we had 4 students pursue a semester project to investigate 4 different detection methods in different laboratories. The aim was to narrow down the options until the best method was developed and as the same time gain useful knowledge on existing biosensor assays. Our first choice was a technique using nano-plasmonics but unfortunately, at the end of the summer semester, it didn't seem effective and implementable in a portable device and we opted for a promising technique from a lab with which we were able to negotiate collaboration terms.

Since the very beginning of our journey, we have collaborated with Abionic, a local start-up active in the area of point-of-care allergy and sepsis diagnostics. Abionic has expressed interest in the sensor we have been building. We have also contacted a cardiologist for advices as we felt important to truly understand the needs of the practitioners and the peculiarities of the Swiss market when it comes to insurance reimbursements of diagnostic tests. Finally, we got in touch with the Technology Transfer Office of our university and had the possibility to discuss our technology and business model with the organizers of their Tech/Business Case club that aims at examining new interesting technologies and their go-to-market strategy.

4. Translation Potential

4.1. Health application potential

In modern healthcare, in vitro diagnostics do not simply tell a doctor whether a patient has a certain disease or not. Our vision is that cardiologists will leverage the available IVD tests potential to make the right decisions for their patients at the right time for effective management and prognosis of the disease. On the long-term, the development of personal diagnostics will allow people to monitor their health and do so in the convenience and privacy of their own homes or in a resource-limited setting.

In order to understand the current needs of health professionals better, we interviewed some experts in the medical area. In their professional opinion, having a sensor in NT-proBNP does not give sufficient information on the patient's cardiac health [6],[10]. Cardiologists rather use echocardiography for diagnostics. Moreover, existing point-of-care tests for NT-proBNP detection (Alere Triage, Ramp by ResponseBio, etc.) already offer a convenient alternative to detect the biomarker in the range of interest for heart failure. Therefore, we have looked into possible ways to innovate our value proposition. Our aim is to develop a sensor that can detect several of the significant markers of cardiovascular disease, including NT-proBNP, but also serum electrolytes concentrations such as sodium and potassium ions (which can increase due to heart failure medication such as ACE inhibitors [9]) and creatinine in whole blood. This would allow the physician and patient to get a more complete overview of the health state and complement more traditional diagnostic procedures. Since the MITOMI technique is capable of multiplexing and has already been used to characterize protein-DNA, protein-RNA, protein-protein and protein-small molecule interactions [1], these tests could be done simultaneously with the same blood sample amount. Taken twice a month, this test would allow to carefully track and manage the progression of heart failure and related conditions.

Marching together with the 4th Industrial Revolution, we think it is important for the future to have the possibility of integrating the results from our test to the electronic medical records of a patient. Test results would be stored in a database and accessible with patient's consent to the doctors.

The advantages of our device with current practice can be therefore summarized as follows:

1. Shorter time-to-result
2. Device weight similar to what's on the market, but portable and miniaturisable
3. Easy-to-use with regards to: loading the sample and the detection solution
4. Scalability and multiplexing with the same sample are possible, reducing time to results, amount of antibodies needed
5. Software with a growing database of results for improved precision of readings using machine learning algorithms
6. Software adaptable for integration with smart hospital systems

4.2. Industrialization and commercialization

For our product, we have the following opportunities: on a social scale, there is the growing fraction of geriatric population and growing incidences of cardiovascular diseases. From a technological point of view, there is ongoing research on cardiac biomarkers, and ongoing clinical trials for the identification of novel cardiac biomarkers, which are driving the growth of the cardiac marker testing market. There is also a rise in the adoption of nanotechnology-based medical devices, and increased nanotechnology R&D expenditure, which is favourable for microfluidics devices. From an economic perspective, there is increasing funding from public-private organizations for research on cardiac biomarkers, a high demand for point-of-care and rapid diagnostic techniques (the market for cardiac market testing IVD is estimated to reach USD 3.5 billion by 2021 [13]).

5-year cycle

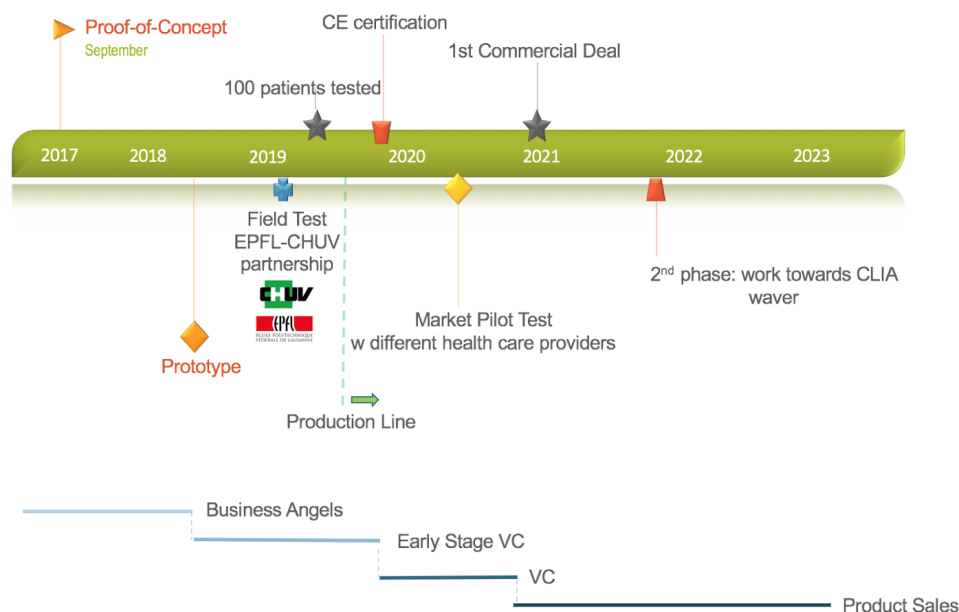


Figure 6 : Commercialization Timeline and Vision

We plan on transforming our lab proof-of-concept into our first prototype by mid-2018. This would allow us to carry out a field test leveraging our University network with a local hospital. The tests obtained from this field trial would serve as the basis to apply for CE certification to be able to bring our device to the Swiss market and/or apply for equivalence using the 510(k) pathway. In 2019 we plan on constructing a first product line to start leveraging economies of scale before commercializing our device in 2021. In the second stage, we plan on adapting our device to be suitable for auto diagnostics (as opposed to use by practitioners until then) and apply for a CLIA

waver. The bottom part of the chart demonstrates possible funding means. We estimate the total costs of bringing our device to the market to be EUR 50 mil.

Our Pricing Strategy & Business Model:

Based on a competitive benchmarking analysis, we estimate the price of our device and service as follows:

- SwisSense machine: 1000 CHF
- 25 Test chips: initial price at 800CHF
- Calibration solution: free with chips purchase

The price will be reviewed when more tests will become available on a single chip. Additional revenue sources will be the analytics software and synchronisation of data with patient records, which will have 2 versions.

- A basic free version: shows the read-out in an exportable format
- Paid subscription: to be negotiated with health providers.

For the paid version, the displayed result is also compared to the results & diagnostic outcome database and generates a personalised message to aid the decision-making. We also offer guidance on how to integrate the results with patients medical records and systems already in place at the healthcare provider.

Miniaturization potential and foreseen economies of scale:

We plan on building a version of the device with an integrated set-up with a raspberry pi instead of a computer. Such a version would be particularly suitable for settings with limited resources or space (e.g. ambulances). We also believe that mass production would reduce the price for PDMS chips and we plan on adapting the pump capacity closer to our needs.

5. Team and Support

5.1. Contributions of team members

Vesna Bacheva: surface functionalization, microfabrication, bio-tests

Remo Blum: electronic setup, mechanical setup, assembly

Hugo Dupont: pneumatic, image processing, microfabrication

Mehdi Gadiri: microfluidic chip design, microfabrication

Vera Glukhenkaya: translation potential, business aspects

Josephine Pratiwi: labelling techniques, translation potential

5.2. People who have given support

Prof. Philippe Renaud & LMIS4 lab members: brainstorming & scientific advice, facilitated access to labs, clean rooms facilities and material ordering.

Prof. Sebastian Maerkl: authorised us to use his patented technique and has continuously advised us during the project.

Dr. Francesco Piraino & Dr. Francesca Volpetti who acted as mentors, taught us basics of the MITOMI technique and were an unconditional source of support throughout the project.

Darja Dubravcic: administrative support and coordination of activities

Evgenii Glushkov: accompanying PhD, gave support on the fluorescence based detection

Dr. Maria Soler: supervisor of one of the initial semester projects; gave support for biochemistry related questions and the nanoplasmonics.

Dr. Jessica Sordet-Dessimoz: head of the histology facility at EPFL that helped us work on the surface chemistry and the sandwich immunoassay optimisation.

Dr. Régis Menétrey (FMH cardiologist), gave us insights into the requirements of cardiologists in Switzerland, insurance-related questions and other practical questions

Konstantinos Kaloulis: Business development advice regarding patents and definition of value proposition from the Technology Transfer Office, Organizer of the Tech/Business Case Club

Geert Leenders: Clinical Research Associate at Quintiles IMS, has shared some insights on the clinical testing stages for a medical device

5.3. Sponsors

Abionic (abionic.com): Abionic is a Swiss start-up specialising in the development and commercialization of point-of-care allergy tests using microfluidics. On numerous occasions, they shared their experience regarding the miniaturization of the lab set-up and commercialization strategy.

Palmos (<https://www.palmos.co>): Palmos is a young American start-up active in the area of sensors for smart cities that has successfully secured fundings in new venture competitions. Palmos Business Developer advised us on how to prepare an effective pitch presentation, reviewed with us the short and long-term development strategy and brought new perspectives from the world of sensors outside of the biomedical field.

Vice-Presidency for Innovation (<http://vpi.epfl.ch/>) and the Technology Transfer Office at EPFL: VPI and the TTO advised us on our patent approach and finance planning.

6. Final remarks

The choice of a detection method based on fluorescence in a microfluidic chip was presented using the MITOMI principle. By having a freestanding membrane that can be actuated by applying a differential pressure, the surface of molecules can be protected from molecules be used for the surface patterning and limit cross-contamination.

The main advantage of our technique is that it is very versatile and can be applied to many healthcare applications. While still at developing stages, it has a lot of potential as a point-of-care device that will meet the needs of the practitioners in the cardiology field and beyond.

Our core technology is already patented and we are ready to enter negotiations with antibody manufacturers to agree on terms for potential commercial use. Our team and advisors combine engineers, experienced entrepreneurs and the main intended user of our device - a cardiologist, which allows us to have a multidisciplinary approach to every problem and most importantly, to ensure that our solutions are relevant and fit with the needs of both patients and doctors!

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Appendix

Information on MITOMI Patent [11]:

MITOMI is a micro-mechanical method recently developed to allow the quantitative analysis of molecular interactions (Maerkl and Quake, 2007). MITOMI consists of a freestanding "button" membrane, which can be actuated by pneumatic or hydraulic pressure, similarly to standard micro-mechanical valves generated by multilayer soft-lithography. When the button membrane is actuated it physically contacts a circular area on the glass surface of the microfluidic device. When the button membrane is in contact with the glass surface it protects the surface from solute and solvent. MITOMI can be used to mechanically trap surface bound molecules between a surface and the button membrane, preventing dissociation of these molecules and thus allowing the measurement of transient molecular interactions. A MITOMI analogue high-throughput microfluidic platform for the quantification for antigen has been described (Volpetti et al., 2015). In this device, primary antibodies are isolated in individual detection regions by MITOMI to eliminate cross-talk between individual assay units of the device. The primary antibody binds a specific analyte, which is then recognized by a fluorescently labelled secondary antibody. The primary antibody/analyte/secondary antibody complex is trapped by the button membrane. The analogue detection signal produced by binding of the fluorescently labelled secondary antibody is quantitated.

Today, diagnostic tests are an integral part of the clinical decision making process along the entire continuum of a patient's health related topics, enabling physicians to make full use of in vitro diagnostics along the healthcare value chain. In vitro diagnostics have been influencing over 60% of clinical decisions. Diagnostic testing empowers doctors to make the right decisions for their patients at the right time for the effective management and prognosis of disease.

Information on Microfabrication [14]:

Regardless of the optical system used and the system of pumps that can be purchased, the essential element that needs to be manufactured is the microfluidic chip. We need to have multiple layers (one for the valve, one for the channel) that are soft since one of them is actuated through pressure variation. Multilayer Soft lithography can then be used to fabricate microfluidic channels, enabling the bond of multiple layers. Molds need to be fabricated on silicon wafers with standard photolithography to create the control and flow channels. The schema of the workflow can be seen in the Figure 7.

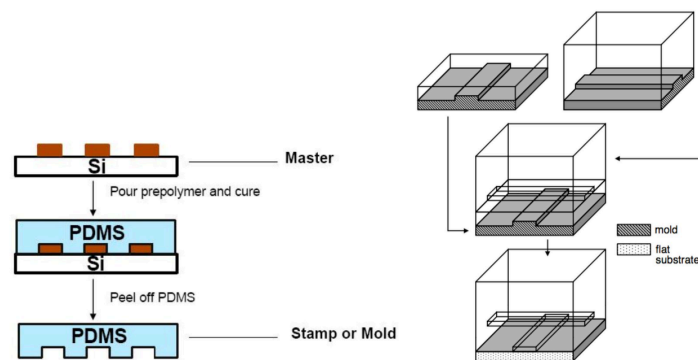


Figure 7: Schematic of the workflow

As for the material, elastomers such as Polydimethylsiloxane (PDMS) are soft materials that can deflect with only small forces. PDMS is a two component addition-cure silicone rubber. The fluidic channel has an excess in curing agent whereas the actuation channel has an excess of the other component, enhancing adhesion between the layers. Since they are monolithic (same material), internal stress is avoided.

The layers can be bonded using plasma bonding and access holes can be easily done through the PDMS for the fluidics and the connection to the pumps.