

Development and control of microfluidic systems

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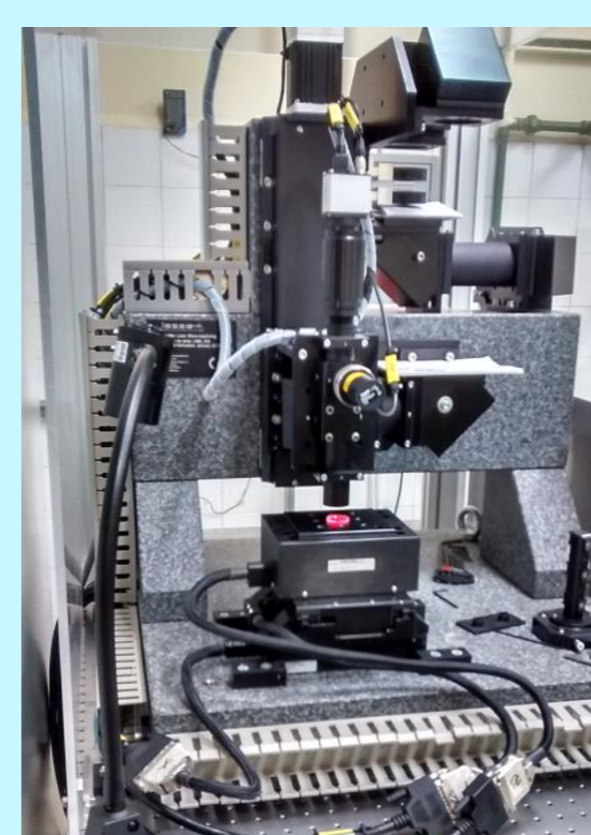
Abstract: The first stage of the project was to perform tests for controlled and optimized machining of BK7 optical glass by femtosecond laser. Parameters such as energy, overlapping pulses, and the focal position variation were investigated for a better extraction of the material. Microchannels, microvalves, micropumps, mixers, reactors, heaters and other components were developed to compose applied microfluidic systems. Microchannels built in BK7 glass separated by a PDMS film form the basis of the microfluidic circuits. The reagent flow control is done by pneumatic mini-valves controlled by an Arduino microcontroller through a Labview platform. This work shows the components developed and two microfluidic systems created. The first contains a microfluidic circuit capable of replicating enzyme-linked immunosorbent assays (ELISA) with a much lower cost of materials. The second has a microfluidic circuit for the production of NaYF₄ fluorescent nanocrystals specially used as markers in images of biological systems.

Introduction: The field of microfluidics has encouraged the development of miniaturizing laboratory equipment and processes. These processes proffer minor reagent consumption, automation, compactness, and cost effectiveness. Time of experimentations may be drastically reduced, from hours for typical immunoassays realized in microtiter plates to minutes with microchannels¹. Microfluidic devices with integrated microvalves show promise for the automation of multiple, parallel, and/or sequential chemical processes on a single chip under digital control². We developed basic microfluidic components to be incorporated into applied systems and two microfluidic systems, one for enzyme-linked immunosorbent assay (ELISA) where we described an assay with Jararaca antigen and another for the production of NaYF₄ nanocrystals that demonstrate the effectiveness of the systems.

Laboratory: A 3-axis CNC station coupled to the equipment with femtosecond laser technology is used for machining of microchannels in BK7 optical glass.

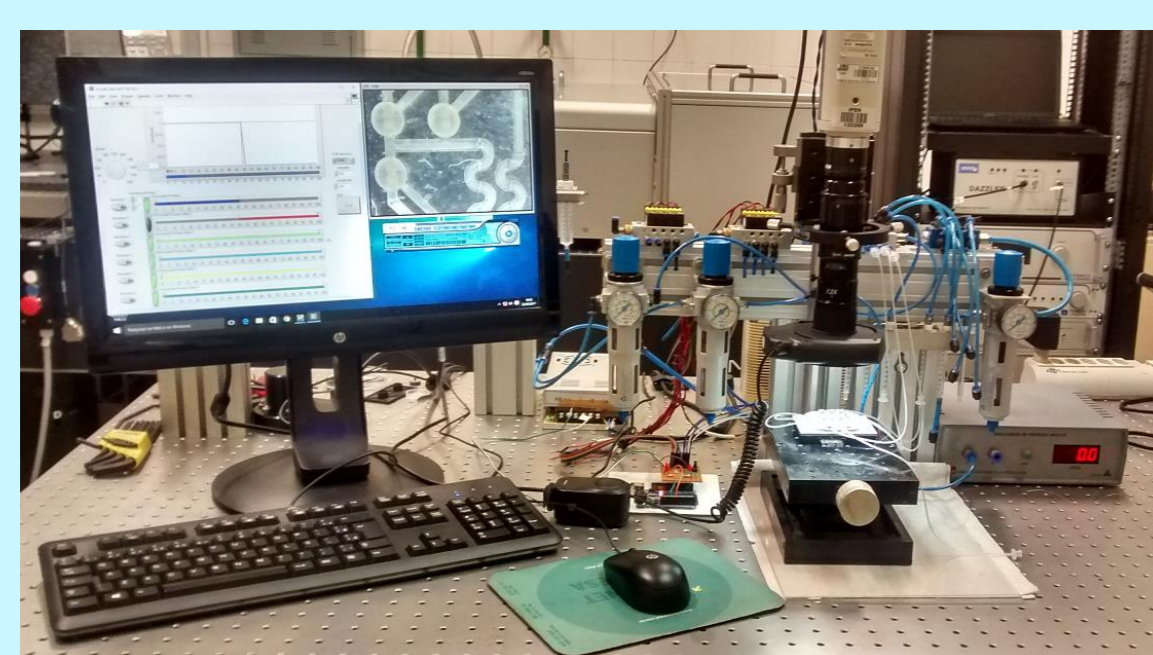


ThorLabs Laser Equipment

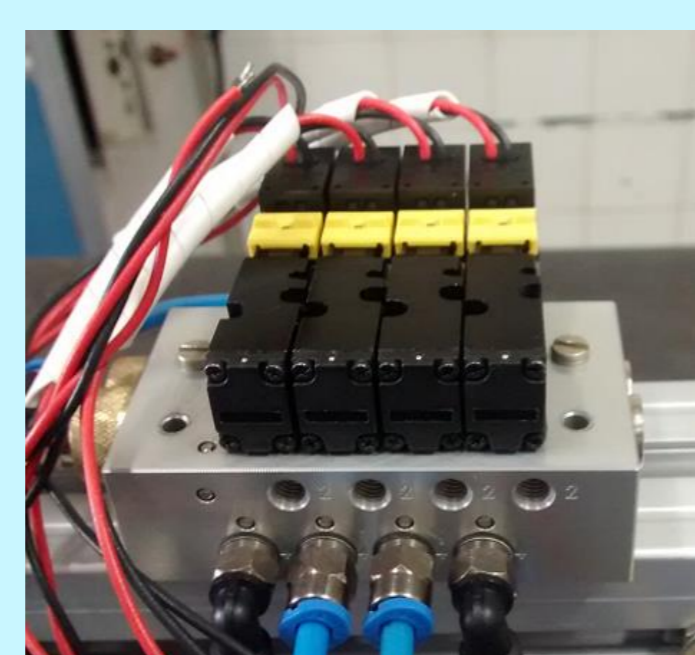


3-axis CNC Station

Workstation: The workstation has a pressurized air inlet, filter and pressure regulator units, reagent storage system, pneumatic mini-valves, Arduino microcontroller and camera coupled to a computer for monitoring.



Work station with camera coupled



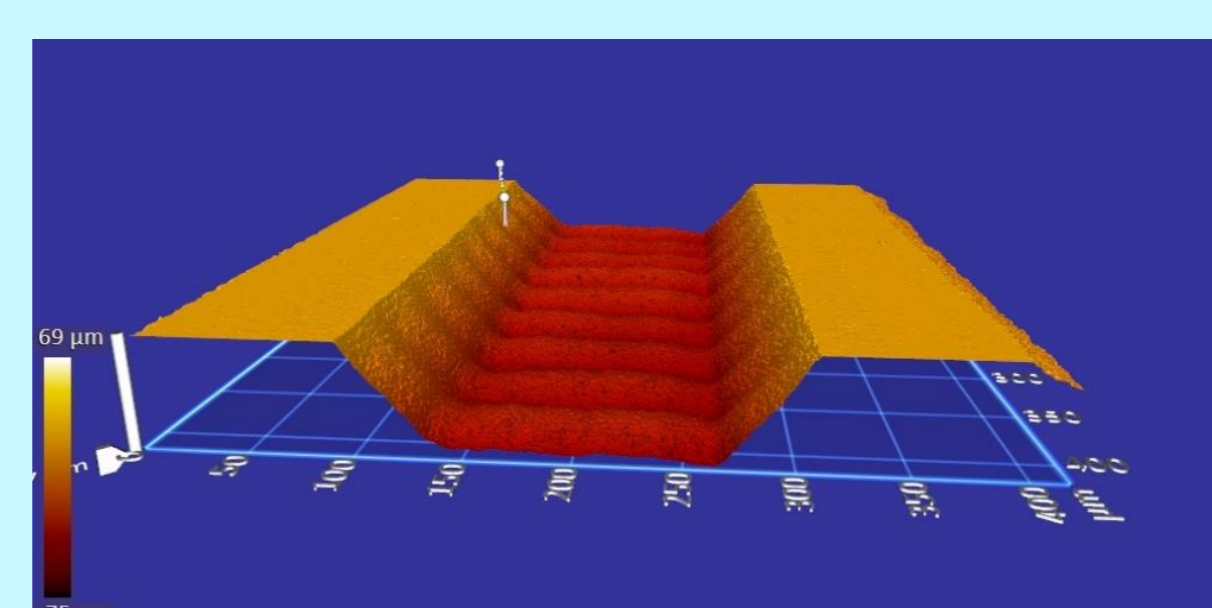
Pneumatic mini-valves



Reagent storage system

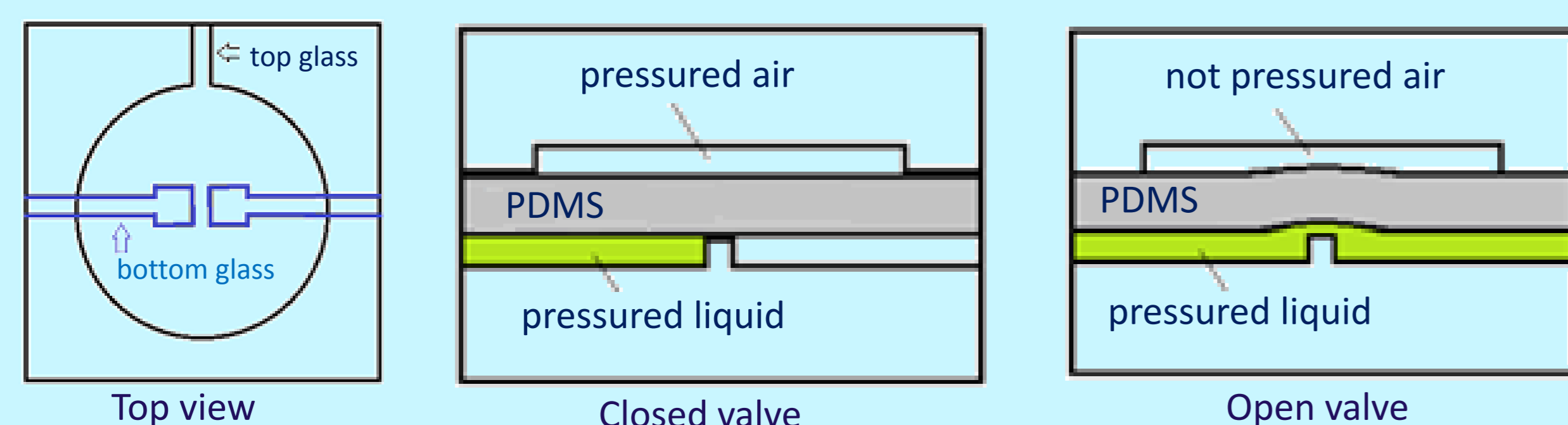
Microfluidic Components: Several components were development to realize some functions into the microfluidic systems.

1. Microchannel: Numerous assays were performed to optimize the machining of microchannels with dimensions of a few hundred widths with depths controlled from 20 μm to 400 μm. Parameters such as energy, overlapping pulses, and the focal position variation were investigated.

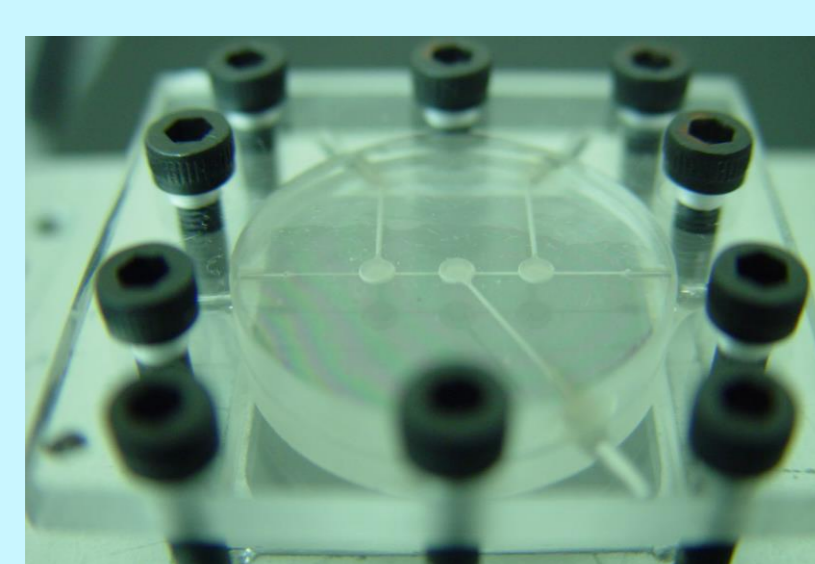


Optical profilometry of a machined channel with optimized parameters.

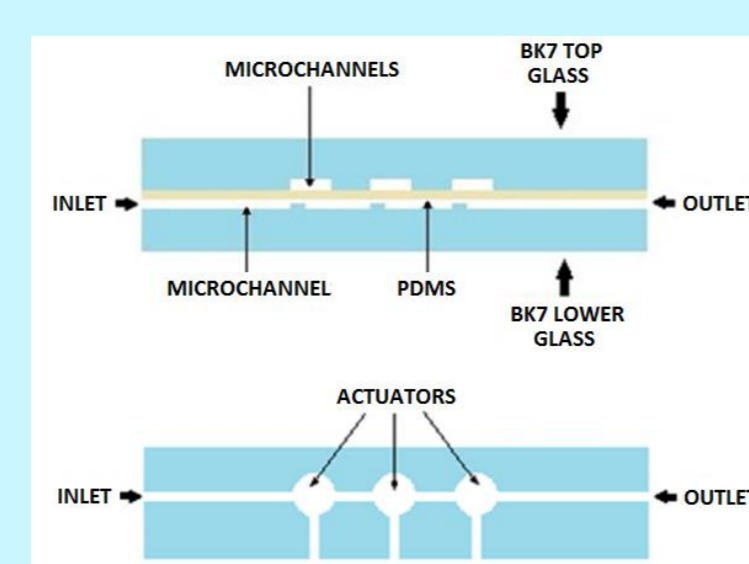
2. Microvalve: The flow of the reagents is controlled by acting on the pneumatic mini valves through the Arduino microcontroller. Toggling the presence or absence of pressure in the upper channels, flows from tens of nanoliters to hundreds of microliters can be obtained.



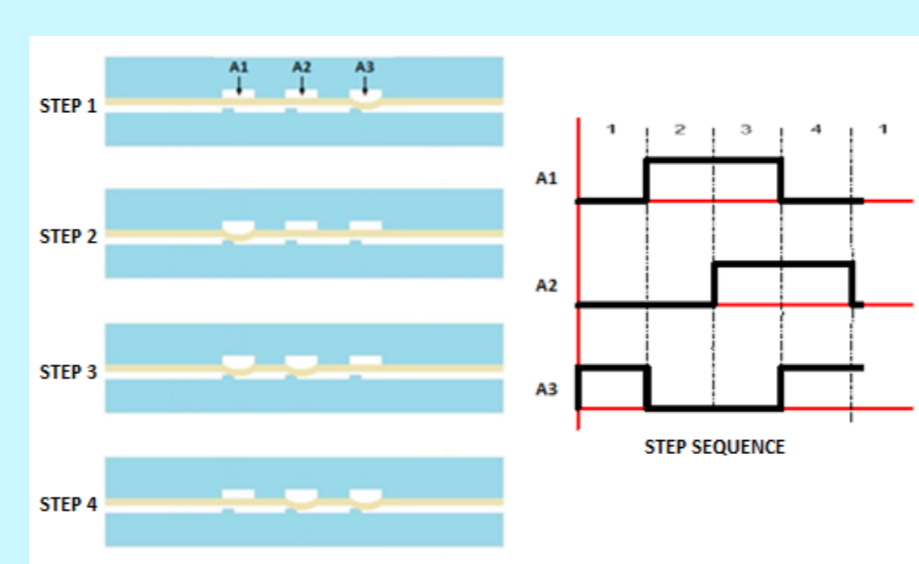
3. Micropump: In some closed circuits where the reagents need to be mixed and it is not possible to inject pressure externally to push the fluids a micropump is required. The micropump constructed by peristaltic effect is described below and the switching sequence that gave the best result is shown.



Micropump circuit

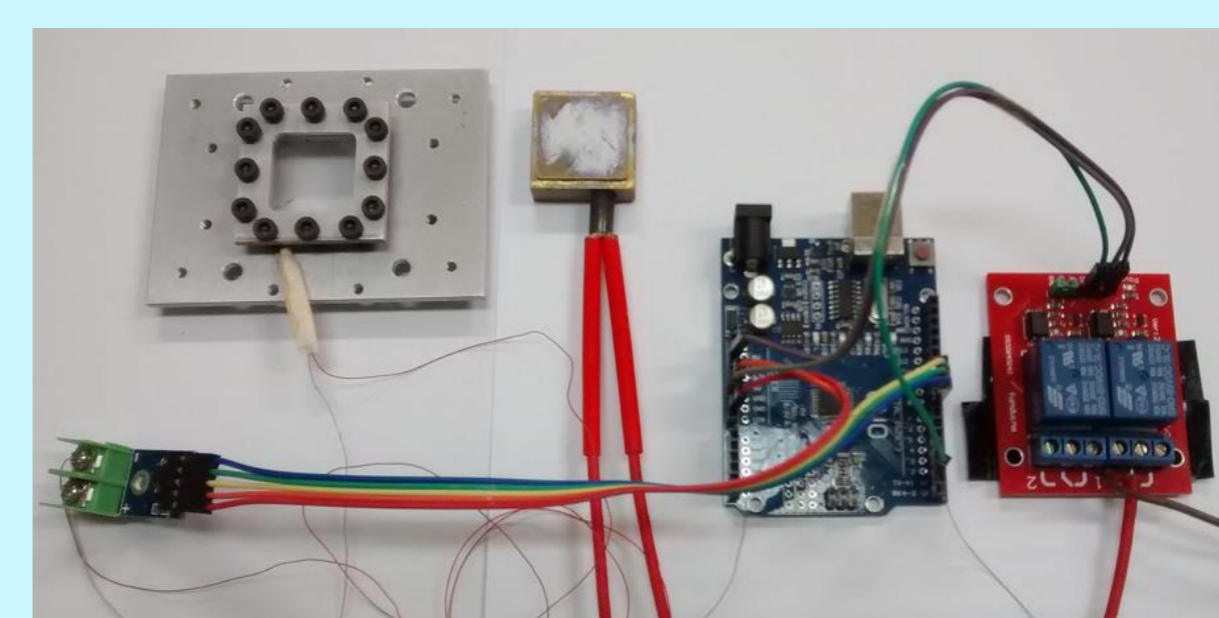


Micropump scheme

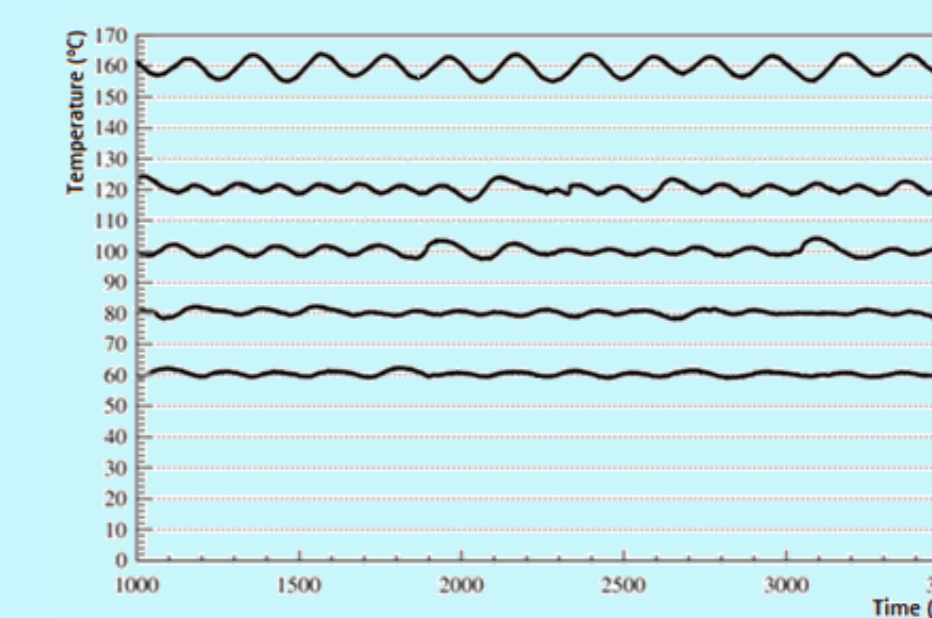


Step sequence

4. Heater: The heating system has a thermocouple placed between the PDMS and the bottom substrate of the circuit that provides the temperature value to the Arduino microcontroller. It acts on a relay turning on or off the voltage in the heating cartridge (electrical resistance) to achieve and control the set temperature.



Components to temperature control

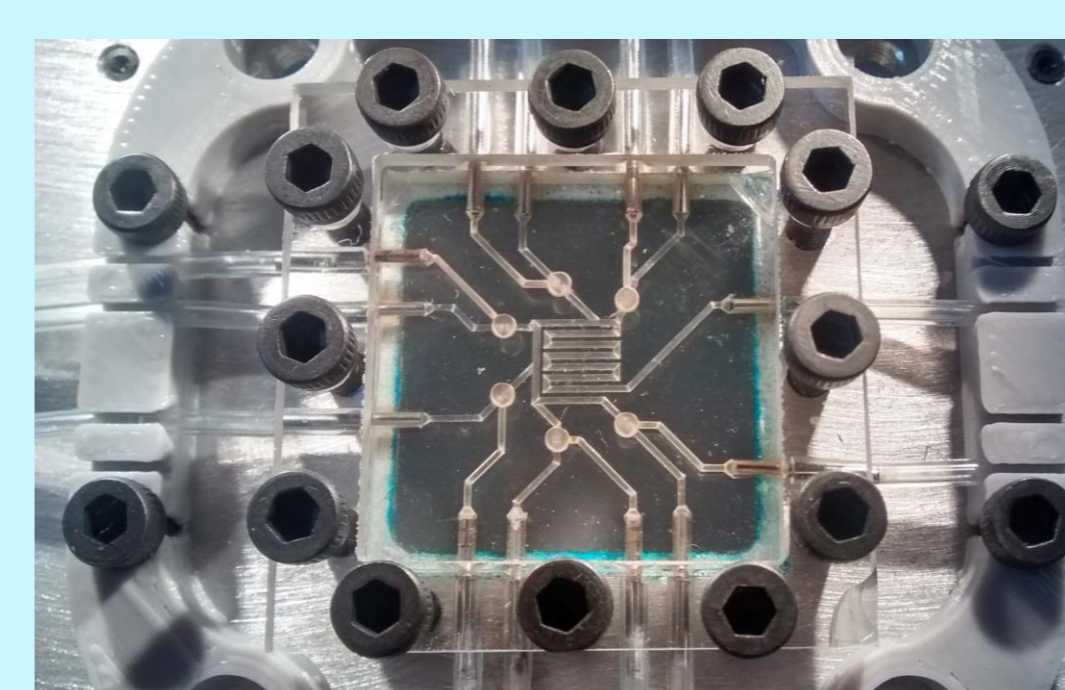


Temperature control for different set-points.

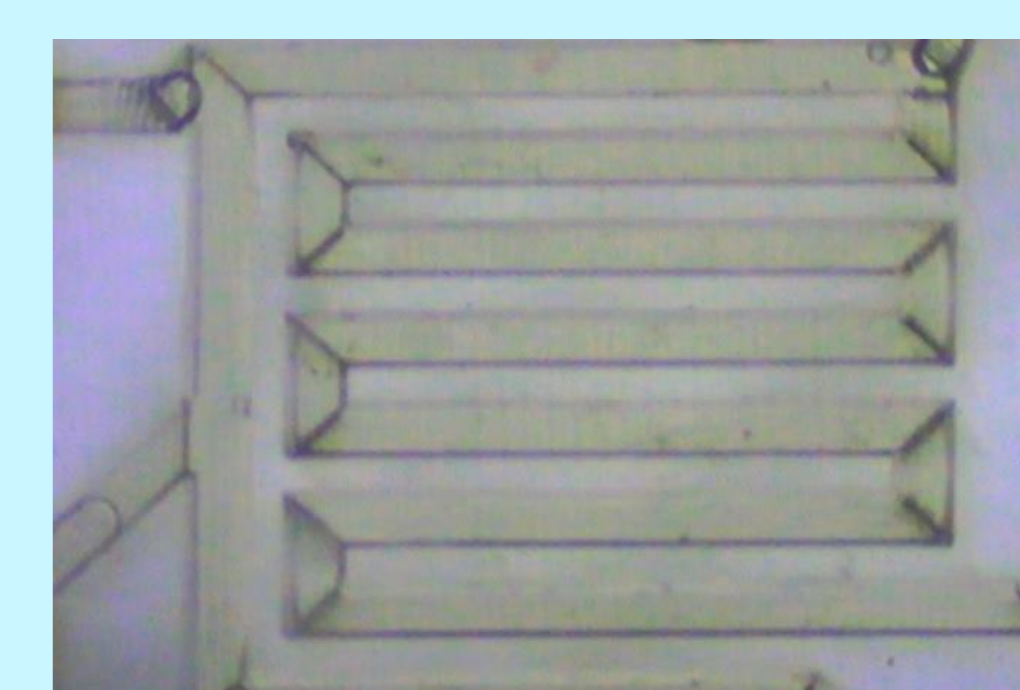
ELISA Microfluidic System : The microfluidic circuit is composed of 6 liquid inlets and 1 outlet. The reagents are described in the table below:

Syringe	Reagents
1	Jararaca atigen (100 μg/ml diluted in TBS)
2	TBS – tris-buffered saline, pH 8 (wash solution)
3	3% skim milk diluted in TBS (blocking solution)
4	Antibiotropic serum of Butantan, 1:5000 in TBS (primary antibody)
5	IgG anti-horse IgG (whole molecule) - peroxidase antibody, 1:5000 (secondary antibody)
6	Orthophenylenediamine (1 mg/ml in TBS with 0.15% H ₂ O ₂)

- Process**
- The circuit was sensitized overnight with jararaca antigen (1).
 - The microreactor was then washed with TBS (2) to remove non-adsorbed antigen.
 - The blocking solution (3) was injected in the reactor with the purpose of adhering in spaces in which the antigen did not adhere.
 - The circuit was washed with TBS (2) for 4x to remove non-adhered milk.
 - The next step was the inoculation of primary antibody (4) that remained in the circuit for 40 min and after was washed with TBS (2) for 4x.
 - Then, the secondary antibody (5), which is responsible for the reaction, was placed and remained incubated in the loop for 40 min. After this time, was washed with TBS (2) 4x.
 - In order to occur the colorimetric reaction the orthophenylenediamine solution (6) was injected into the loop and the color change (transparent to yellowish) was observed in approximately 20 min.

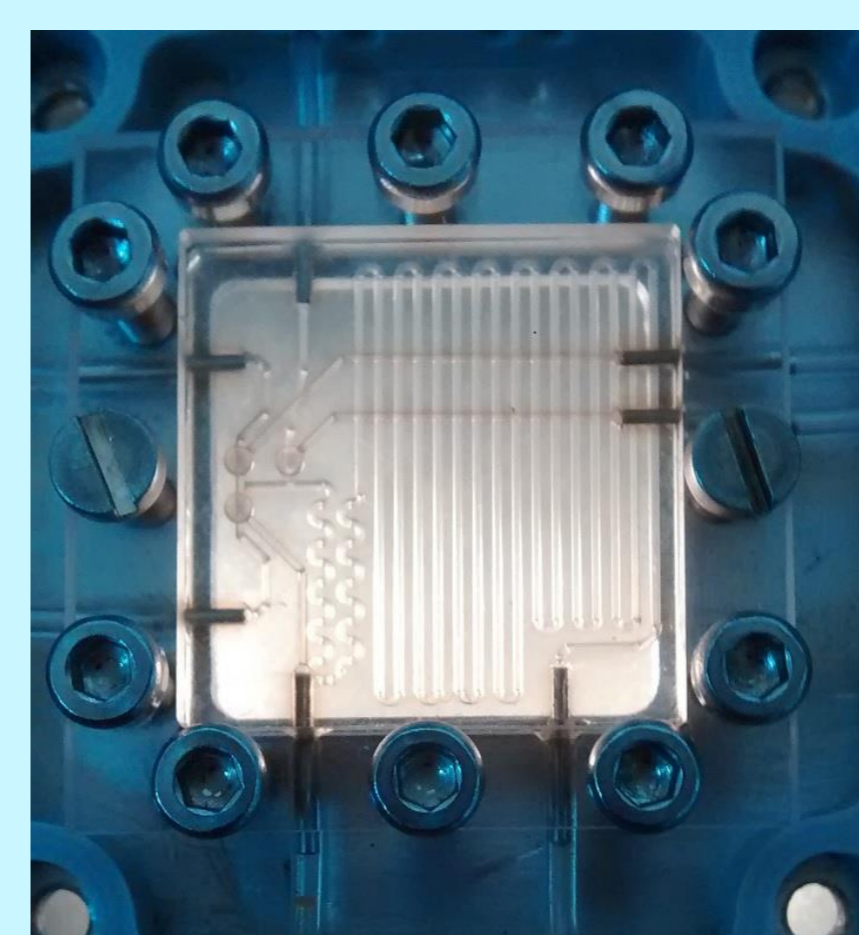


ELISA Microfluidic System



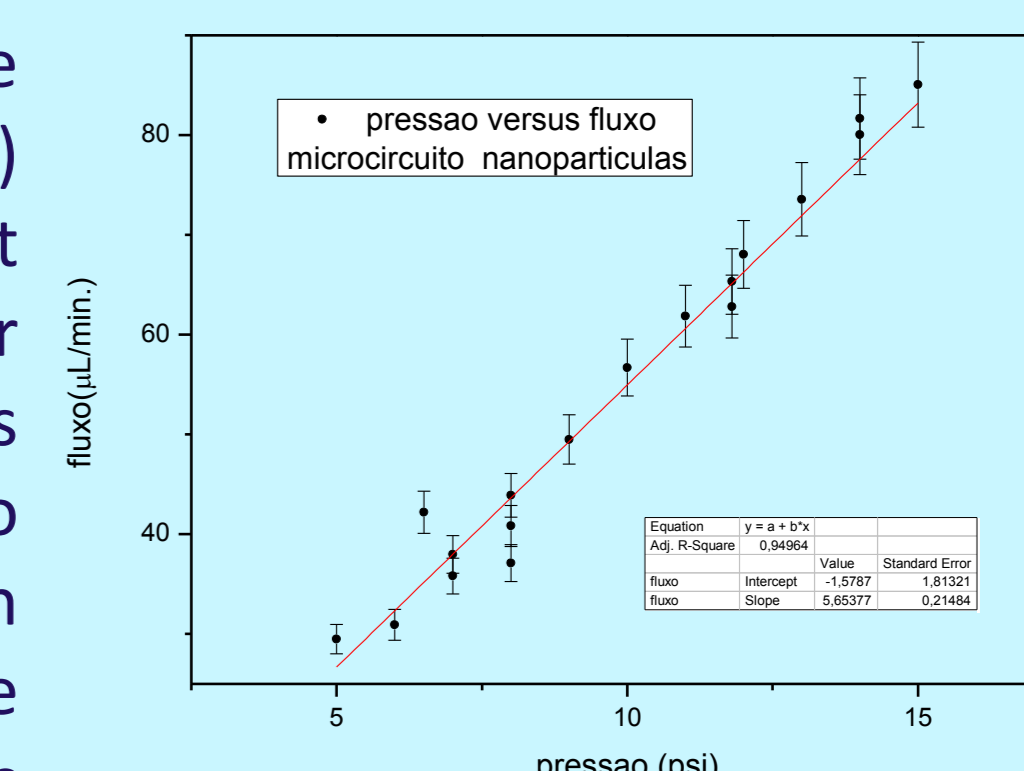
Yellow color indicating the presence of jararaca antigen.

NaYF₄ fluorescent nanocrystals : The microcircuit has 2 inlets for reactants and 1 air inlet and 1 outlet to collect the nanocrystal. It has an initial stage for mixing and a serpentine to increase the residence time. The figure below shows the microfluidic system.



Nanocrystal Microfluidic System

Injection of reagents in this system is controlled by the pressure (compressed air) exerted on the inlet and outlet valves. In this way a prior calibration of the pressures versus flow was necessary to size the desired compositions in the synthesis processes. The graph obtained having water as the fluid, is shown in figure on the right



pressures versus flow - water

References:

- [1] DUBUC, D., CHEN, T., CHRÉTIENNOT, T., POUPOT, M., FOURNIÉ, J. Microfluidic On-Chip for Biomedical Applications. *IEEE*, p. 129-132, 2011.
- [2] LEE, C.C., SUI, G.D., ELIZAROV, A., SHU, C.Y.J., SHIN, Y.S., DOOLEY, A.N., HUANG, J., DARIDON, A., WYATT, P., STOUT, D., KOLB, H.C., WITTE, O.N., SATYAMURTHY, N., HEATH, J.R., PHELPS, M.E., QUAKE, S.R., TSENG, H.R. Multistep synthesis of a radiolabeled imaging probe using integrated microfluidics. *Science*, v. 310, p. 1793-1796, 2005.

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